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Scientific Output

Articles
Proceedings
Books
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Invited Oral Communications
Oral Communications
Poster Communications
PhD Thesis
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Awards
About BERG

2011
BERG Annual Report
The BioEngineering Research Group (BERG) celebrated 20 years in 2011, fostering the development of biochemical engineering and life sciences in the fields of industrial, and health biotechnology and bioenergy. This year BERG expanded the location of its research laboratories to the IST campus at Taguspark, with the inauguration of a premier research laboratory in the emergent area of Stem Cell Bioprocessing.

This report highlights the activities of the five research thrust areas and laboratories of BERG within the Associated Laboratory Institute for Biotechnology and Bioengineering.

The major activities in the Bioprocess Engineering and Biocatalysis Laboratory included the development of technologic platforms for faster development of fermentative/bioconversion processes, from micro- to pilot-scale in the field of White Biotechnology and of biosensors and microfluidic platforms for monitoring and control of bioprocesses in the areas of environment, food, water and health-care.

The Bioseparation Engineering Laboratory developed novel purification processes in order to intensify and optimize the downstream processing of proteins and biopharmaceuticals, with special emphasis on monoclonal antibodies (mAbs), with main focus on aqueous two-phase extraction, nano-magnetic separation and monolithic chromatography, from a nano-scale to industrial scale.

The main research topics at BioSystems Engineering Laboratory were focused on: i) new or established Process Analytical Technology (PAT) tools, ii) whole process/product design and analysis (cell-process-product), iii) systems engineering applied to modern manufacturing and iv) pharmaceutical engineering.

The Nucleic Acid Bioengineering Laboratory addressed the scientific/technological challenges associated with plasmid biopharmaceuticals by combining biomolecular engineering studies with bioprocess engineering and to co-develop (with INESC-MN) thin-film microchip and microfluidic platforms for the manipulation/detection of DNA, proteins and cells, through the development of: i) plasmid vectors and their application in gene therapy or DNA vaccination; and ii) microchips for DNA detection.

The Stem Cell Bioengineering and Regenerative Medicine Laboratory focused on the ex-vivo expansion of stem cells and their controlled differentiation into specific cell types for Cellular and Gene Therapy and Tissue Engineering, through the development of highly controlled bioreactor systems and advanced bioseparation and purification techniques.

Joaquim M.S. Cabral
BERG Head and Director of IBB
The BioEngineering Research Group (BERG) is a research unit in engineering and life sciences at the Centre for Biological and Chemical Engineering (CEBQ). CEBQ is the leading Centre of the Associated Laboratory Institute for Biotechnology and Bioengineering (IBB), a network of research centres across Portugal. IBB has been identified by the Portuguese Ministry of Science, Technology and Higher Education as a strategic infrastructure for the development of the Portuguese R&D and innovation policies in the areas of Biotechnology, Bioengineering, Life, Biomedical and Agricultural Sciences. BERG activities within the Associated Laboratory IBB are focused on the Thematic Areas of Industrial and Environmental Biotechnology/Bioenergy, Health Biotechnology and Nanobiotechnology.

BERG aims at excellence in research and advanced education in biotechnology and bioengineering. The overall goal is to contribute for a better understanding of the mechanisms that occur at the molecular and cellular levels, in order to translate them into rational applications of biological systems relevant to the Industrial and Health care sectors. BERG research priorities have special emphasis on Bioprocess, Biosystems and Biomolecular Engineering, Gene/Nucleic Acid Bioengineering, Nanobiotechnology and Stem Cell Engineering, featuring an integrated cross-disciplinary approach through five laboratories:

- Bioprocess Engineering and Biocatalysis Laboratory (BEBL)
- Bioseparation Engineering Laboratory (BEL)
- BioSystems Engineering Laboratory (BSEL)
- Nucleic Acid Bioengineering Laboratory (NABL)
- Stem Cell Bioengineering and Regenerative Medicine Laboratory (SCBL)
BERG was established in 1991 as one of the initial three research groups of the Centre for Biological and Chemical Engineering at IST, under the coordination of Prof. Joaquim Sampaio Cabral. The research carried out throughout the years has been considered of excellent level by the international committees, which regularly evaluate the research units funded by the Portuguese Ministry of Science, Technology and Higher Education. The main output of the activities performed by BERG members in 2011 is summarized in the following tables.

**Human Resources**

In 2011, BERG has 90 researchers integrated into the five laboratories, of these 28 have a PhD degree, 33 a master degree, 1 a five-year diploma degree, 25 a bachelor degree and 1 undergraduate.

<table>
<thead>
<tr>
<th>Human Resources</th>
<th>Number</th>
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<tr>
<td>Faculty</td>
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<td>Research Scientists</td>
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<tr>
<td>Post-doctoral Fellows</td>
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<td>PhD Students</td>
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<tr>
<td>MSc Students</td>
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<tr>
<td>Research Assistants</td>
<td>9</td>
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<tr>
<td>Technicians</td>
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</table>

**Publications**

In 2011, the output of BERG’s activities includes the publication of 45 scientific articles in peer-reviewed journals, 2 books and 6 book chapters, among other publications, including conference proceedings, invited oral communications, oral communications and poster presentations in distinct international and national conferences.
### Facts and Numbers

<table>
<thead>
<tr>
<th>Type of Publication</th>
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<tr>
<td>Papers in international peer-reviewed journals</td>
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<tr>
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<td>Patents</td>
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<tr>
<td>Invited oral communications in international conferences</td>
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<tr>
<td>Invited oral communications in national conferences</td>
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<tr>
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<tr>
<td>PhD Thesis</td>
<td>8</td>
</tr>
<tr>
<td>MSc Thesis</td>
<td>37</td>
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### Scientific events

In 2011, the members of BERG have participate in organizing and scientific committees of national and international conferences, research networks, working groups and sections of the European Federation of Biotechnology and of the Tissue Engineering and Regenerative Medicine International Society (TERMIS).

<table>
<thead>
<tr>
<th>Type of Event</th>
<th>Name of Event</th>
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<tr>
<td>Symposium</td>
<td>Organising Committee “Stem Cells and Cellular Therapy in Cardiovascular Diseases – Portugal”, July, Lisbon</td>
<td>Joaquim Cabral</td>
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<tr>
<td>Conference</td>
<td>Scientific Committee of International Conference on BioPartitioning and Purification (BPP), September, Puerto Vallarta</td>
<td>Raquel Aires Barros</td>
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<tr>
<td>Conference</td>
<td>Scientific Committee of the 19th Biennial Meeting of the International Society for Molecular Recognition (Affinity), June, Tavira</td>
<td>Raquel Aires Barros, Ana Azevedo</td>
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<tr>
<td>Conference</td>
<td>Scientific Committee of International Meeting of the Portuguese Society for Stem Cells Therapies (SPCE-TC) Braga, Portugal</td>
<td>Joaquim Cabral, Cláudia da Silva, Margarida Diogo</td>
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<tr>
<td>Conference</td>
<td>Scientific Committee of the 4th Joint National Congress of Microbiology and Biotechnology (Microbiotec11) December, Braga</td>
<td>Joaquim Cabral, Raquel Aires Barros</td>
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<td>Working Group</td>
<td>Scientific Committee of TERMIS Thematic Group on “Bioreactor Technologies”</td>
<td>Joaquim Cabral</td>
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<tr>
<td>Working Group</td>
<td>Scientific Committee of European Section on Applied Biocatalysis “ESAB”</td>
<td>Joaquim Cabral, Luís Fonseca</td>
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<td>Working Group</td>
<td>Downstream Processing of the European Section of Biochemical Engineering Science “ESBES”</td>
<td>Raquel Aires-Barros</td>
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<td>Workshop</td>
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<td>Joaquim Cabral</td>
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Research Activities

2011
BERG Annual Report
**Objectives**

The Bioprocess Engineering and Biocatalysis Laboratory aims at developing competitive and sustainable technologic platforms and analytical methodologies and tools with high potential and economic impact. In the field of biocatalysis the main goal is to design and produce value-added bioproducts by bioconversion using enzymes and microbial cells from micro- to pilot-scale in the field of White Biotechnology, namely in key areas such as of food and feed, aroma, pharmaceutical and fine chemistry industries, and biofuels, as well as to improve biocatalyst performance. A second area is the development of new analytical methodologies and devices specially biosensors and microfluidic systems designed for monitoring and control of bioprocesses, environment, food and water and healthcare.

The current projects are focused on the development of technological platforms for biocatalysis and analytical tools organized in three major areas: i) Biocatalysis and Biotransformation, ii) Biosensors and Miniaturization, and iii) Bioenergy.

**Research Topics**

1. **Biocatalysis and Biotransformations** - Design and thorough characterization of reaction media and operational strategies aiming at the implementation of robust, high conversion bioconversion systems is performed. These are anchored in enzymatic platforms, namely cutinase, penicillin acylase and inulinase, targeted for the production of esters (flavors, biodiesel, chiral compounds and glycerol and oil intermediates for bioplastic production), antibiotic intermediates and semi-synthetic antibiotics and sweeteners. New methodologies on the use of lipases in miniemulsion systems have been used on the enzymatic resolution of secondary alcohols with economical interest. Bio-degradable zwitterionic compounds are being synthesized in order to be used as drug delivery vehicle. Moreover, the work developed contributed with valuable insight towards the definition of major guidelines for rational bioprocess design and development. Whole cells of mesophilic bacteria have been improved to be able to carry out biocatalytic and bioremediation processes under extreme conditions of temperature and pH and in the presence of high concentrations of salt and copper.

2. **Biosensors and Miniaturization** – Nano/micro-biocatalysts (biocomposites) are being developed based on hydrogels, sol-gel, protein/cell assemblies and magnetic nano-particles. New biomaterials compatible with enzymes and other bio-molecules are being used as matrices on the development of biosensors. Miniaturized platforms, viz. miniature, meso- and microreactors are used for bioprocess intensification. Reliable scaling strategies, from those platforms to, at least bench-scale, is looked into. Use of microtiter plate platforms for high throughput screening of given biocatalytic activity and for the early stages in the development of fermentation/bioconversion process were developed. High throughput systems were also used to test the ability of essential oils from aromatic Mediterranean plants to prevent biofilm formation and to study cellular adaptation mechanisms to different toxic compounds. A method involving bacterial cells was used as a non-destructive technique to detect micro defects in microfabrication components.

3. **Bioenergy** – Enzymatic (viz. lipase, cutinase) and whole cell platforms (yeast strains) are being used within the scope of an innovative approach for the sustainable production of biodiesel and jet biofuel. Further research efforts are being made towards the production of jet-fuel within the scope of microbial cell factories. Oxido-reductases are used in combination with an innovative material, Ion Jelly, for structuring enzymatic biofuel cells. *Rhodococcus* cells are being used for micro-production of electricity and as a source of fatty acids for biofuel production.
Main Achievements

• High throughput platforms were advantageously used for fast and thorough characterization of heterogeneous bioconversion systems targeted for specific applications, namely inulin and cellobiose hydrolysis, clearly fastening the pace of process development.

• The enzymatic production of intermediate therapeutic steroids was implemented in a microfluidic platform under aqueous-organic two phase systems.

• Optimized synthesis of flavor compounds anchored in miniemulsion systems, an environmentally friendly approach.

• Development of a microtiter plate platform for the high throughput evaluation of acetylcholinesterase inhibitors.

• The enzymatic resolution of several secondary alcohols has been achieved in good yields and excellent enantiomeric excess.

• New biomaterials have been applied on the development of simple and cheap glucose paper test strips. These glucose paper test strips presented a quick response, less than one minute, good morphological and functional stability in physiologic solution at 37°C for a period up to one hour. The effect of parameters such as maturation and sweling on the preparation of biomaterials has been studied.

• A new class of zwitterionic compounds has been prepared and studied as potential electrolytes. The conductivity measurements of these compounds have shown very good and promising results. Biodegradable zwitterionic compounds have been prepared with the aim to be put together with different drugs and improve the drug delivery.

• The bioproduction of siderophores was scaled-up from a high throughput platform used to screen suitable bacterial strains.

• Mesophilic *Rhodococcus erythropolis* cells were adapted to work under extreme conditions. The adaptation of bacterial cells to toxic compounds and adverse environments was determined using an integrated approach using fluorescence microscopy techniques, membrane composition and cell wall properties.

• The antimicrobial properties of okra extracts were determined and the essential oils of Portuguese aromatic plants were used as inhibitors of cell adhesion and biofilm formation.

Selected Publications


Coutinho, C.P., de Carvalho, C.C.C.R., Madeira, A., Pinto-de-Oliveira, A., Sá-Correia, I., Infection and Immunity, 79, 2950-2960


de Barros, D.P.C., Azevedo, A.M., Cabral, J.M.S., Fonseca, L.P., J. Food Biochem., 58, 545-556


Marques, M.P.C., Fernandes, P., Molecules, 16, 8368-8401

Objectives

The Bioseparation Engineering Laboratory aims at the design and development of novel purification processes in order to intensify and optimize the downstream processing of proteins and biopharmaceuticals, with special emphasis on monoclonal antibodies (mAbs). Several alternatives to the currently established downstream processing platforms of recombinant proteins are being explored, with main focus on aqueous two-phase extraction, nanomagnetic separation and monolithic chromatography, from a nano-scale to industrial scale. Additionally, tailor-made synthetic ligands are being used aiming to improve protein purification, stability and function.

Research Topics

1. Aqueous two-phase systems (ATPS) for biopharmaceuticals purification – The feasibility of using aqueous two-phase extraction as a general platform for the purification of biopharmaceuticals, especially of mAbs is being studied. The performance of a pilot scale packed differential contactor for the continuous countercurrent aqueous two-phase extraction (ATPE) of human immunoglobulin G (IgG) from a Chinese hamster ovary (CHO) cells supernatant is being evaluated and compared to the batch IgG extraction. The economical and environmental sustainability of an ATPE based capture process is been evaluated and compared to the currently established platform. Efficient models are being developed in order to predict protein partition in ATPS, contributing for a better understanding of the mechanisms responsible for partitioning of biomolecules and the parameters governing partition. A novel cell separation process based on immunoaffinity aqueous two phase systems to isolate and purify human hematopoietic stem/progenitor cell directly from the whole umbilical cord blood (UCB) is being developed in collaboration with SCBL.

2. Bio-inspired affinity polymer systems for antibody recognition – Novel biomimetic affinity nanoparticles, based on the conjugation of a Protein L-mimic affinity ligand with thermosensitive amine-functionalised PNIPAM microgels, were designed and synthesised. Adsorption screening with three different model-proteins (bovine serum albumin, a commercial monoclonal antibody (mouse IgG, isotype) and human IgG) demonstrated that such bio-inspired nanoparticles are able to selectively recognize and capture antibody molecules in both pure and impure/complex media.

3. Nano-magnetic separation of biopharmaceuticals – The main focus is on the development of magnetic nanoparticles suitable for the purification of mAbs. The feasibility of using boronic acid functionalized magnetic particles in the adsorption of mAbs under conditions typically observed in mammalian cell culture is being evaluated and compared with analogous supports coated with Protein A.

4. Monolithic chromatography for integration of cell separation and antibody purification – Novel affinity and mixed-mode ligands for the purification of mAbs, with particular focus on phenyl boronate derivatives, have been designed and developed. The immobilization of the ligands on to the surface of supramacroporous monoliths (cryogels) will allow the integration of both clarification and capture in just one step, and thus the capture of mAbs directly from cell culture media without any cell removal step upstream.

5. High throughput bioseparation platforms – A lab-on-a-chip device is being designed and tested for mAbs extraction using ATPS in a microfluidic platform, as an effective tool to accelerate bioprocess design and optimization. The partition of IgG tagged with fluorescein isothiocyanate in ATPS is being investigated in a PDMS microfluidic device fabricated using soft lithographic techniques in collaboration with INESC-MN and BEBL. Process simulation will predict IgG diffusion and partitioning behav-
Main achievements

• The suitability of a packed differential contactor for the ATPE of human antibodies from a CHO cells supernatant has been shown. Higher IgG recovery yields and purities were obtained when compared to the batch IgG extraction.

• The economical and environmental sustainability of an ATPE based capture process has been successfully evaluated and compared to the currently established platform (Protein A). The ATPE process has shown to be considerably advantageous in terms of process economy and operation, especially when processing high titer cell culture supernatants. This alternative process is able to purify continuously the same amount of mAbs reducing the annual variable operating costs by at least 39% when cell culture supernatants with mAb titers higher than 2.5 g/L are processed.

• PEG/dextran/NaCl aqueous two-phase system (ATPS) was successfully used for the specific partitioning and recovery of CD34⁺ stem/progenitor cells from UCB. Purification factors up to 245 were achieved with a single step partitioning experiment, demonstrating the feasibility of using ATPS as an alternative step to the traditional techniques for UCB processing.

• The feasibility of using phenyl boronate as an alternative ligand to protein A for the direct capture of mAbs from clarified cell culture supernatants has been demonstrated. Boronic acid magnetic particles provided higher binding capacity and identical affinity towards IgG when compared with magnetic particles coated with Protein A. Complete recovery of bound IgG was achieved after optimization of the elution conditions. Considering the substantially lower cost and higher stability at alkaline conditions of the boronic acid, this synthetic ligand could be an alternative to Protein A.

Selected Publications


Objectives

To link process and product throughout design, development and biomanufacturing, systems engineering approaches must be used or developed anew. Process analytical technology (PAT) represents the combined use of different tools applicable through many of those stages. Though PAT is still mostly process centred, it can be used within the QbD (quality by design) context to link process to product. The main research topics at BioSystems Engineering Laboratory (BSEL) are focused on: i) work with new or established PAT tools, ii) whole process/product design and analysis (cell-process-product), iii) systems engineering applied to modern manufacturing, and iv) pharmaceutical engineering.

Research Topics

1. Process Analytical Technology Tools – PAT involves the application of process analytical chemistry (i.e., in-process monitoring techniques and chemometrics), multivariate data analysis (MVDA; e.g., data-based modelling techniques), and process control techniques (namely, use of process data with multivariate supervision and diagnosis strategies). All these activities are done with the aim of characterizing the state of a system at any given time real-time and to be used in process optimization and control. The perspective taken in PAT is that of the process (not the sample or that of a single parameter over time). In this research topic the use of spectroscopy techniques specially suited for industrial applications is explored in diverse contexts (pharma/biopharma) and within the overall PAT context and aims.

2. Whole Process/Product Design and Analysis – Systems biology and ‘omic’ approaches have provided a general physiological and metabolic engineering understanding of several important microorganisms, while bioprocess systems engineering has benefited from the integration of monitoring, modelling, control and optimization. In this topic the links within and between USP (up-stream processing: biotransformation) and DSP (down-stream processing: bioseparation) are explored at the three levels of cell-process-product. Concepts such as process and product design spaces and all aspects related to quality-by-design are examined for pharma and biopharma processing.

3. Systems Engineering Applied to Manufacturing – While product innovation has been the key issue in pharma and biopharma in the past, manufacturing has remained relatively static (e.g., locked-validated processes). Continuous improvement and operational excellence practices are entering pharma/biopharma manufacturing and transforming these industries as happened elsewhere decades ago. In this research track, science and technology driven manufacturing paradigms are the main topics examined, as the way forward to achieve operational excellence and sustainability throughout process/product life-cycle. Adapting tools and metrics from the disciplines of operation excellence in other industries to biomanufacturing, is the main focus on this topic.

4. Pharmaceutical Engineering – New paradigms in design and manufacturing of small and large therapeutically active (API) molecules and drug products (formulated APIs), include continuous microreaction technologies (MRT). It is relatively straightforward to scale-down and operate in continuous mode some API chemical synthesis reactions in micro-reactors. It is still very complex or yet unfeasible to operate in continuous mode most unit operations involving suspended solids (e.g., crystallization), biotransformations and some bioseparations. In this research topic work with off-the-shelf MRTs is being initiated to examine both feasibility and operability issues of plant miniaturization and process intensification of small API molecules manufacturing. As experience and knowledge are established more complex types of products and unit operations will be examined.
Main Achievements

• Developing and demonstrating the industrial feasibility for GMP monitoring of near-infrared spectroscopic for at-line multiparametric monitoring large biomolecules’ manufacturing processes with scale, clone and media independent calibrations.

• Whole process analysis with PAT techniques in pharma and biopharma manufacturing.

• Proposing a general framework for developing and applying QbD through process analytical technology tools across diverse problems and platforms.

• Developing new algorithms for data fusion and improved information extraction of PAT monitoring tools.

• Implementing microwave resonance to pilot and industrial scale pharmaceutical granulators for in-situ PAT monitoring and QbD studies.

Selected Publications


Nucleic Acid Bioengineering

Duarte Miguel Prazeres (PI), Gabriel Monteiro, José Santos, M. Ângela Taipa, Marília Mateus

Objectives

Nucleic Acid Bioengineering Laboratory (NABL) is focused on: i) plasmid vectors and their application in gene therapy or DNA vaccination; and ii) microchips for DNA detection. The specific objectives are to address the scientific/technological challenges associated with plasmid biopharmaceuticals by combining biomolecular engineering studies with bioprocess engineering and to co-develop (with INESC-MN) thin-film microchip and microfluidic platforms for the manipulation/detection of DNA, proteins and cells.

Research Topics

In the case of plasmids, the following research topics are pursued:

1. Design, stability and delivery of plasmids – Parental plasmids are designed to improve the manufacturing of minicircles. Marine organisms are screened for drugs that inhibit nucleases, stabilise plasmids and hence lead to higher transfection activity. Delivery systems (electroporation, liposomes, carbon tubes, polymeric microparticles) are developed to increase DNA uptake and transcription levels.

2. Manufacturing of plasmid vectors - Processes for the production of plasmids are conceptually designed, developed, optimised and compared. E. coli strains are engineered to produce high amounts of plasmid DNA by mutating key genes on the glycolytic pathway. The impact of the downstream processing on the overall quality and biological activity of plasmids is studied. Downstream processes are combined with strain engineering and parental plasmid design to facilitate the purification of minicircles. Membranes are designed to improve plasmid chromatography. Covalent immobilization of plasmids and assembling of molecular probes on AFM cantilevers is pursued to characterize binding interactions with membrane adsorbers. Synthetic protein-mimic affinity ligands are screened and used to purify plasmid DNA. Analytical procedures (HPLC) to monitor manufacturing and product quality are also developed.

3. DNA vaccines and gene therapy – DNA vaccine candidates are constructed by cloning antigenic proteins associated with sleeping sickness/avian flu and tested in mice models for their ability to generate cellular and humoral responses, and to provide immunisation. The possibility of using plasmids to deliver the cytotoxic bacterial protein azurin to cancer cell models is under evaluation (collaboration with BSRG).

In the case of microchips for DNA detection the following topics are addressed:

1. Immobilization and handling of DNA proteins and cells – Thin film technologies, chemical modification, microfluidics and electronic addressing are used to develop microchips for the molecular recognition of specific analytes via hybridization. The core of the chips is a flat surface with immobilized probe molecules or cells. Other features include the presence of micro-electrodes to generate electric fields that accelerate the kinetics of binding/recognition.

2. Photodetectors – Amorphous silicon photodetectors are developed for the optoelectronic detection of coloured, chemiluminescent and fluorescent molecules in thin film chips. The presence of these molecules ultimately reports specific biorecognition events such as DNA hybridization or metabolic cell activity.

Main Achievements

- The role played by charge transfer interactions in the clearance of cell-derived impurities (RNA, DNA, proteins, lipopolysaccharides) from plasmid-containing E. coli lysates by phenyl boronate chromatography at acidic pH was described.
• *E. coli* strains that produce high amounts of plasmid DNA were created by systematically mutating key genes on the glycolytic pathway.

• A parental plasmid for minicircle production was constructed by inserting two identical MRS sites and the gene for ParA resolvase in a commercial eukaryotic expression vector pVax.

• The critical influence of the downstream processing on the ability of plasmids to form lipoplexes and transfect mammalian cells was demonstrated.

• Liposome-immobilized membranes were developed and their feasibility as HIC adsorbers in a plasmid DNA downstream purification protocol was assessed.

• The surface chemistry of several membrane adsorbers was determined by X-ray photoelectron spectroscopy (collaboration with CQFM-IST).

• Microfluidic systems were developed to carry out microspot-based ELISA in with chemiluminescence and colorimetry detection using integrated thin-film amorphous silicon photodiodes (in collaboration with INESC-MN).

• Label-free electrical detection of surface DNA immobilization and hybridization via streaming current measurements in a microchannel was demonstrated.

**Selected Publications**


Novo, P., Prazeres, D.M.F., Chu, V., Conde, J.P., Lab-on-a-chip, 11, 4063-4071

Objectives

The Stem Cell Bioengineering and Regenerative Medicine Laboratory aims at developing highly controlled bioreactor systems for the ex-vivo expansion of stem cells and their controlled differentiation into specific cell types, as well as their integration with advanced bioseparation and purification techniques. As stem cells (SC) are rare, their isolation and expansion/differentiation in vitro significantly increases the cell population available for Cellular and Gene Therapy, Tissue Engineering, high-throughput drug screening and stem cell research. Human hematopoietic stem cells (HSC), human mesenchymal stem cells (MSC), as well as human and mouse pluripotent stem cells (both embryonic (ESC) and induced pluripotent stem cells (iPSC)) and neural stem cells (NSC) are used as model systems.

Research Topics

1. Ex-vivo expansion of HSC in co-culture with MSC under serum-free conditions - Current research is focused on: (i) the definition of optimal culture conditions namely concerning cytokine combinations, enrichment procedures and initial cell concentrations used to provide an amplification of HSC, especially those obtained from the umbilical cord blood (UCB); and (ii) the understanding of the mechanisms underlying the hematopoietic supportive capacity of MSC. These will have implications in terms of bioreactor design towards the maximization of human HSC expansion in vitro. Current research also focuses on platelet production from the ex-vivo expanded HSC. Isolation and purification methods of human hematopoietic stem/progenitor cells are being developed in collaboration with BEL, to obtain highly enriched cell populations at large-scale.

2. Clinical-scale production of MSC for Cellular Therapies - Culture protocols are being optimized for the isolation and expansion of MSC under serum-/xeno(geneic)-free conditions, while maintaining their multilineage differentiation and immunosuppressive capacities, as well as their genetic stability. MSC are isolated from adult bone marrow (BM), adipose tissue (AT), umbilical cord matrix (UCM) and synovial membrane. Culture of MSC in fully controlled bioreactors using microcarriers, under defined, xeno-free conditions, is currently being exploited to maximize MSC yield. In addition, a proteomic analysis platform is being established in collaboration with BSRG, IBB/CEBQ, to understand how the ex-vivo culture process affects MSC features at the proteome level.

3. Bioprocessing of pluripotent and neural stem cells - The ex-vivo expansion of pluripotent stem cells (PSC) and PSC-derived NSC is studied towards the definition of highly controlled bioreactor systems to establish an efficient, reproducible and cost-effective large-scale bioprocess to obtain the starting material to generate mature cells (i.e. neurons) for potential use in the treatment of neurological disorders, as well as for drug screening. Bioseparation and purification methods of human PSC-derived cells are addressed to ensure the quality control for cellular therapies.

4. Micro-Scale culture of pluripotent stem cells - High-throughput microarray systems, as well as microfluidic devices are being developed to elucidate important microenvironmental factors (i.e. chemical, physical) affecting mouse and human pluripotent stem cell self-renewal and differentiation, while providing the basis for rapid identification of signals and conditions that can be used to direct cellular responses.
5. Gene delivery strategies to stem cells - Safe and effective non-viral strategies to genetically engineer stem cells are being developed to enhance the therapeutic efficacy in different clinical settings. DNA vectors encoding for reporter and/or specific proteins involved in ex-vivo expansion/differentiation of stem cells are being delivered to these cells by microporation or associated to cationic lipids. Novel gene carriers such as minicircles and miniplasmids are currently being exploited, in collaboration with NABL, to extend gene expression and augment cell survival and proliferation, foreseeing the maximization of stem cells for applications in Cellular and Gene Therapy, as well as Tissue Engineering.

6. Tailoring biomaterials to support stem cell cultivation - Synthetic polymeric supports are developed to assist scalable culture systems for maximization of ex-vivo stem cell expansion or differentiation. Electrospinning is currently being used to produce nanofiber scaffolds to mimic aspects of the extracellular matrix, promoting cell-cell and cell-material interactions and cellular adhesion. Moreover, some biomedical applications require cell recovery from the polymeric support at the end of the cell cultivation stage. Polymers sensitive to harmless stimuli (e.g. glucose, temperature) are being tailored to release cells, without affecting cell viability and function, at physiologic conditions.

Main Achievements

- By studying the effect of the initial degree of CD34+ cell enrichment on the expansion of hematopoietic stem/progenitor cells from UCB in co-culture with human BM MSC, it was demonstrated the existence of highly dynamic culture events regarding CD34 modulation, prior to cell division, affecting cell cycle and proliferation status in culture and ultimately the final hematopoietic cell yield. These events point to the need to establish a balance between the cell recovery upon purification and the stem/progenitor cell proliferative potential of cultured cells.

- A novel cell separation process based on an immunoaffinity aqueous two phase system (ATPS) composed of polyethylene glycol (PEG) and dextran was successfully established to isolate and purify...
CD34+ stem/progenitor cells directly from whole UCB. This system is expected to pave a new way to purify hematopoietic stem/progenitor, at a process scale, for use in a variety of clinical settings.

- The microcarrier-based stirred culture system previously developed for human MSC was successfully adapted to xeno-free conditions. Furthermore, this xeno-free stirred culture system was able to support the expansion of both BM MSC and adipose-derived stem cells (ASC), while maintaining the characteristic immunophenotype and multipotency differentiation potential. These results represent a major step towards the GMP compliant large-scale production of a safe and effective MSC for Cellular Therapy.

- A two-dimensional gel electrophoresis (2-DE) based quantitative proteomic study was performed in collaboration with BSRG, IBB/CEBQ for unveiling the molecular mechanisms underlying the commonly observed decrease on proliferative and clonogenic potential of human BM MSC upon consecutive passages. Proteins of the functional categories “Structural components and cellular cytoskeleton” and “Folding and stress response proteins” were found to be less abundant in later passages, while the levels of proteins involved in “Energy metabolism”, “Cell cycle regulation and aging” and “Apoptosis” were increased. This platform paved the way to establish a proteomic analysis platform as a quality control for MSC products towards the development of safer and more effective cellular therapies.

- Hypoxic conditions (2% O₂ versus atmospheric air) were found to induce an immediate and concerted downregulation of genes involved in DNA repair and damage response pathways in human BM MSC and ASC, concomitantly with the occurrence of genomic instability in microsatellite markers, while maintaining telomere length. These results provide evidence on how hypoxia selectively impacts the cellular response of BM MSC and ASC, thus pointing towards the need to optimize oxygen tension ex-vivo according to the cell source.

- A robust and quality-controlled large-scale culture system, under serum-free conditions, was developed for the mass production of mouse ESC (mESC) in a fully-controlled stirred tank bioreactor. Importantly, cells expanded under these conditions retained the expression of pluripotency markers and their differentiation potential into cells of the three embryonic germ layers. This controlled bioprocess is potentially adaptable to other cell types including human ESC and iPSC, thus representing a promising tool for the controlled production of specific cell types for applications in tissue regeneration and drug screening.

- A multifactorial design approach was successfully used to elucidate the sole and interactive influence of different signaling pathways in the regulation of the effect of oxygen tension towards mESC expansion under chemically defined conditions. MEK/ERK pathway inhibition, activation of Wnt/β-Catenin by GSK-3 inhibition and activation of STAT3 were evaluated. These results add new insights into the mechanisms by which oxygen tension influences mESC fate with GSK-3 inhibition showing a crucial role towards maintenance of mESC pluripotency under a low oxygen tension.

- A microcarrier-based culture platform was developed for scaling-up the expansion of both mouse and human PSC-derived NSC, under adherent serum-free conditions, in spinner flasks and using xeno-free microcarriers. This culture system was able to support PSC-derived NSC expansion while maintaining their neural stem/progenitor phenotype and neuronal differentiation potential.

- A feeder-free and serum-free culture platform was successfully established for the expansion of human iPSC under static conditions allowing the maintenance of the pluripotency phenotype after a successive sub-culturing procedure. This platform encompasses completely xeno-free culture conditions and a single-cell passaging methodology towards a more accurate control and characterization of human iPSC cell expansion.

- Novel DNA vectors devoid of bacterial sequences – Minicircles - were used to genetically engineer human MSC and mouse NSC. The obtained results have shown that stem cells transfected with these vectors exhibit higher survival and transgene expression, for a longer period of time, using lower amounts of DNA when compared to the respective plasmid. These findings provide evidence for the advantages of using minicircles for over-expressing therapeutic proteins, mainly envisaging clinical applications.
• An electrospinning system was assembled, key parameters adjusted and different collectors built and tested for the production of matrices with different nanofiber alignments. A range of materials, including cellulose acetate, dextran, polycaprolactone and polyhydroxybutyrate were used to produce nanofibers with diameters between 75 and 1750 nm. These matrices were successfully tested as cellular supports for cultivation of human stem cells.

Selected Publications


Research Highlights

2011
BERG Annual Report
Bioprocess Intensification through miniaturization

The versatile *Rhodococcus erythropolis*

Affinity based purification of human monoclonal antibodies from CHO cell supernatants using boronic acid magnetic particles

Economical evaluation of aqueous two-phase extraction as a novel platform in the biomanufacturing industry

Combining microwave resonance technology with multivariate data analysis as a PAT/QbD approach to improve process understanding in pharmaceutical processes

Microchip-integrated photodetection of intracellular calcium in response to the activation of G-protein coupled receptors

Rational engineering of *E. coli* strains for improved manufacturing of plasmid biopharmaceuticals

Controlled mass production of mouse embryonic stem cells in bioreactors

Multifactorial analysis of embryonics stem cell self-renewal reveals a crucial role of GSK-3-mediated signaling under hypoxia
Globalization brought along increased competitiveness, which has further stressed the need for fast development of more cost-effective and sustainable (bio)processes. Process intensification, where large and expensive equipments/processes are replaced with cheaper, smaller and more efficient ones is an acknowledged approach to comply with such demand. The use of miniaturized devices clearly fits within the scope of process intensification, since they require minimal amounts of chemicals and biological; allow for high level of parallelization; and, in given configurations enable scale-out rather than scale-up [1]. Although miniaturization can be implemented from upstream to downstream of a (bio)process, its application in fermentation/bioconversion steps clearly stands out, where it relies in an assorted type of reactor configurations [1,2]. With volumes under 100 mL to a few μL, these reactors may or may not display a microstructured nature. The former configuration abridges microchannel plate and monolith type reactors, whereas the latter encompasses miniature stirred tanks and microtiter plates (MTP). Miniaturized reactors can be used in different stages of bioconversion/fermentation processing, more specifically during process development or at production scale. Once rationally used, evidence on the advantages of the use of miniaturized reactors in the former stage have been increasing; on the other hand, and despite some examples of successful applications in production scale when purely chemical processes are involved, the use of microreactors at production scale when biologicals are used, the potential for application is still under evaluation [1,2]. The work developed has contributed to further consolidate the relevance of miniaturized reactors for the early stages of bioconversion process development.

Microfluidic reactors for bioconversion of steroids

The production of intermediate steroids from sterol substrates is a multi-enzymatic reaction. The first step is the conversion of the 3β-hydroxy function into a 3-keto derivative, which is performed by cholesterol oxidase (CO). Given the lipophilic nature of sterol and steroid molecules, the use on non-conventional media, such as organic-aqueous two phase systems, is a common approach to overcome the low volumetric productivity of aqueous bioconversion systems [3]. Moreover, the use of microfluidic reactors when enzymatic catalysis requiring transport across phase boundaries is clearly favored, due to the enhanced mass transfer typical of said microreactors. Since the selected bioconversion yields as by-product H$_2$O$_2$, which may deactivate CO, a second enzymatic reaction was added, involving catalase (CAT), resulting on H$_2$O$_2$ decomposition (Fig.1).

The assembled set-up comprised a Y-shaped microfluidic reactor for cholesterol oxidation coupled to a packed bed mesoreactor, where polyvinyl alcohol (PVA) immobilized CAT decomposed H$_2$O$_2$. The microfluidic reactor operated in a heptane-phosphate buffer environment, where the organic phase was a pool for substrate and product. Hydrogen peroxide, dissolved in the aqueous phase, was pumped through the packed bed reactor (Fig.2).

The whole allowed for continuous operation over 300 h, where despite the decay of catalytic activity of CO, an overall production of 36 M of cholestenone is expected (Table 1).

Fast characterization of immobilized enzyme systems

**Figure 1.** Two-step enzyme reaction. The oxidation of cholesterol is catalyzed by cholesterol oxidase (CO). The by-product hydrogen peroxide is decomposed by catalase (CAT).
Experimental set-ups, anchored in either batteries of temperature controlled, miniature stirred reactors (under 2 ml volume) or in microtiter plates (MTP), coupled to high throughput analytical methods, preferably anchored in spectrophotometry. These methods, were established. These can be easily adapted for the fast characterization of enzyme or whole cell bioconversion systems. Such setups were used for the characterization of a sol-gel immobilized inulinase [5] and for the rational screening of strategies for the immobilization of β-glucosidase [6]. In the former case, the biocatalyst formulation never reported at the date, was used for the hydrolysis of inulin to fructose. The porous xerogel particles of about 10 µm size, displayed an immobilization efficiency of 80%. As a result of immobilization, activity was displayed over a broader range of temperature and pH. Furthermore, immobilization did not tamper with the native enzyme structure, although it brought along some mass transfer limitations [5]. Still, the sol-gel biocatalyst displayed high operational stability, since it was re-used over more than 20 consecutive batch runs, while retaining high conversion yields (Fig. 3).

Using the retention of the catalytic activity following immobilization as starting criterion for the selection of promising supports for β-glucosidase, sol-gel and PVA-base supports were selected [6].

In either case, immobilization did not change the pH/activity profile, but the use of the sol-gel support improved the temperature/activity profile. Immobilization led to enhanced thermal and pH stability. Nevertheless, immobilization brought along mass transfer limitations. Both enzyme formulations displayed operational stability (Fig. 4).

References
**The versatile Rhodococcus erythropolis**

**C.C.R. de Carvalho**

*Rhodococcus erythropolis* are able to carry out a wide array of bioconversions and degradations due to a large set of enzymes (e.g. oxidases, epoxidases, dehydrogenases, dehalogenases and hydrolases), allowing the production of commercially interesting compounds and the metabolism of recalcitrant organic compounds [1]. These cells are very hydrophobic as a result of a mycolic acid-containing cell wall, allowing the adhesion of cells to oil/water interfaces and the direct uptake of hydrophobic compounds such as hydrocarbons [2]. *R. erythropolis* can even adapt the fatty acid composition of the cellular membrane, the mycolic acid content and the cell wall permeability as a response to the carbon source [2,3].

**Natural tolerance**

*R. erythropolis* cells are able to degrade aliphatic (Fig. 1) and aromatic hydrocarbons, including benzene, toluene, xylene and ethylbenzene, as well as polyaromatic hydrocarbons such as anthracene [1,4]. The cells present a particular ability to carry out biotransformations and bioconversions in organic-phase systems [5].

When non-adapted *R. erythropolis* DCL14 cells were placed in contact with toluene, 10.5% of the cells were still viable after 1h exposure. However, adapted cells were able to degrade 52.4% (v/v) toluene in *n*-dodecane, toluene being consumed at 10.7 mg/(h mg protein) [6]. *Rhodococcus* sp. cells can even be active under starvation conditions and the degradation of toxic compounds may not be negatively affected by the presence of more easily degradable carbon sources such as *n*-dodecane [7]. The cells are also usually more tolerant to antimicrobials. For example, a fresh extract of *Abelmoschus esculentus* at a concentration of 97.7 mg/mL was sufficient to kill all *Staphylococcus aureus* cells, which is a worldwide source of nosocomial infection, as well as *Mycobacterium*, but was ineffective against *R. erythropolis* [8].

**Adaptation to improve cellular performance**

Extremophiles can grow at extreme values of temperature, pH, ionic strength and metal concentrations, but it may be difficult to find and isolate those possessing the required metabolic activities. On the other hand, *R. erythropolis* cells possess a large number of catabolic activities and may be

![Figure 1](image-url)  
*Figure 1.* Growth rates observed using *n*-alkanes as sole carbon and energy sources at 15 and 28°C.
easily adapted to extreme conditions. The physiological adaptations undertaken by these cells, when exposed to conditions sequentially further away from the optimum growth conditions, allowed the activity of the cells to be maintained at conditions previously sufficient to kill non-adapted cells [9] (Fig. 2). The cells were able to grow and degrade C6-C16 n-alkanes and alcohols at 4-37°C, pH 3-11 and in the presence of up to 7.5% sodium chloride and 1% copper sulphate. The large majority of adapted cells were able to maintain polarization of the membrane under the most difficult conditions tested, to adjust the net surface charge and changed the composition of the fatty acids of the cellular membrane according to the growth condition. Changes in the relative proportion of straight, methyl and cyclopropyl saturated, unsaturated and hydroxyl substituted fatty acids were observed, as well as production of polyunsaturated fatty acids unusual in bacteria.

References


Antibodies for therapeutic applications are a fast growing market with increasingly pressing demands. The combination of a large potential market (>500,000 patients) with therapies requiring high doses and/or chronic administration (>1 g per patient per year) served as the driving force towards process optimization [1]. Given the inability of Protein A chromatography to directly purify samples with high monoclonal antibody titers (titers greater than 10 g/l are now possible), alternative and more cost effective purification processes are needed. In this regard, magnetic separations offer fast, gentle and highly selective (non-magnetic impurities) separation conditions with the potential for high binding capacities (small particles, typically < 2 µm). A great effort has been given in the development of fully synthetic ligands to substitute Protein A. Ideally, this ligand would provide selectivity, increased capacity and chemical stability while decreasing the costs. The boronic acid ligand is capable of selectively capturing cis-diol containing molecules, such as carbohydrates and glycoproteins, through the formation of a reversible covalent ester bond. Antibodies are glycoproteins as they bear oligosaccharides in both the Fc and Fv regions. In the former, despite some heterogeneity, the 1,2-cis diol saccharides fucose, manose and galactose can be typically found.

The phenylboronic acid ligand which was used in this work is able to operate as a multi-modal ligand as it is able to promote affinity, electrostatic, hydrophobic, aromatic π-π, charge transfer and hydrogen bonding interactions. Depending on the pH value, the boronic acid moiety might be in a trigonal or tetrahedral form. At pH values lower than the pKa of phenylboronic acid, the trigonal form is predominant and thus charge transfer interactions between the boron sp² empty orbital and any Lewis base (e.g. unprotonated amines) can occur [2]. Conversely, at alkaline pH values, this type of interaction may be disregarded as the boronic acid is converted in to the hydroxyboronate form, which no longer interacts with Lewis bases but is able to promote electrostatic interactions. Contrary to the initial general belief that the optimal pH for the complexation of cis-diol containing molecules with boronic acids is above the pKa of the latter, recent reports demonstrated that this is highly dependent on the molecule-ligand pair used [3,4].

Initial batch adsorption studies with commercially available non-porous silica based boronic acid magnetic particles (SiMAG-Boronic acid) showed the binding pH to be an important factor in the adsorption isotherms of human antibodies (Fig. 1a). The maximum binding capacity was found to

**Figure 1.** Adsorption behavior of human IgG on SiMAG-Boronic acid and SiMAG-ProteinA magnetic particles. A) Human IgG adsorption isotherms of SiMAG-Boronic acid (pH 7.4 ●, pH 8.5 ● and 9.5 ●) and SiMAG-ProteinA (pH 7.4 ■) particles. The lines represent the fitted Freundlich isotherms. B) Adsorption kinetics, q (full symbols) and percentual variation of the binding capacity with time, dq/dt (empty symbols) of human IgG in SiMAG-Boronic acid (circles) and SiMAG-ProteinA (squares) particles at pH 7.4.
be higher at neutral pH than at pH 9.5. Under the same conditions, non-porous silica based Protein A magnetic particles (SiMAG-ProteinA) showed approximately only half of the binding capacity while exhibiting identical affinities to those of SiMAG-Boronic particles at pH 7.4 and 8.5. Furthermore, the adsorption kinetics (Fig. 1b) were found to be very fast with 70% of the maximum binding capacity obtained at 30 min of incubation, being observed in less than 30 s. Such is only possible due to the high affinity of the particles towards the target molecule and to the small size (1 µm) and non-porous nature of the support.

To test the feasibility of using boronic acid magnetic particles as an alternative to Protein A a fully human monoclonal antibody was directly purified from a clarified CHO cell culture supernatant. The most important factor influencing the overall process yield and product purity in boronic acid particles was found to be the binding pH. Basic pH values promoted higher purities while resulting in decreased yields due to the competing effects of molecules such as glucose and lactate present in the cell culture supernatant. After optimization, the particles were successfully used in a multi-cycle purification process of the mAb from the CHO feedstock. Boronic acid particles were able to achieve an average overall yield of 86% with 88% removal of CHO host cell proteins (HCP) when the binding was performed at pH 7.4, while at pH 8.5 these values were 58% and 97%, respectively. In both cases, genomic DNA removal was in excess of 97%. Comparatively, Protein A particles recorded an average overall yield of 80% and an HCP removal greater than 99%. Boronic acid based purification processes can offer a cost-effective alternative to Protein A as the direct capturing step from the mammalian cell culture.

References

Economical evaluation of aqueous two-phase extraction as a novel platform in the biomanufacturing industry


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The biotech industry is currently facing unparalleled challenges due to the increasing demand for biotechnology-based human therapeutic products, such as monoclonal antibodies (mAbs). This has led companies to improve considerably their upstream processes, with production yields increasing to mAbs titers never seen before. The downstream processes have, however, been overlooked, causing a production bottleneck at the downstream level. Although chromatography remains the workhorse of most purification processes, several limitations, such as low capacity, scale-related packing problems, low chemical and proteolytic stability and resins’ high cost, have arisen. Aqueous two-phase systems (ATPS) have shown to be a valuable option for the downstream processing of biopharmaceuticals, combining a high biocompatibility and selectivity with an easy and reliable scale up and capability of continuous operation. In this work, the economical sustainability of the aqueous two-phase extraction process is evaluated and compared to the currently established protein A affinity chromatography (ProA) [1].

The proposed downstream ATPE process is based on a pilot scale validation previously reported by the authors [2], which is depicted in Fig. 1. This ATPE-based capture process consists of three main steps: i) extraction (E), ii) back extraction (BE) and iii) washing (W). In the extraction step, most of the higher molecular weight contaminants are removed, while the washing step allows not only the removal of lower molecular weight contaminants and polymer-rich phase component (PEG), but may also enables the recycling of the polymer for future uses.

The annual operating costs (AOC) required to process 840 m³/year of cell culture supernatant containing 2.5 g/L mAb were estimated to be US$8.7 and 14.4 millions for ATPE and ProA-

![Figure 1. Process flow diagram for the continuous ATPE-based capture of human antibodies from a cell culture supernatant (BP: bottom phosphate-rich phase, TP: top PEG-rich phase, MS: mixer–settler) [2]](image-url)
based capture processes, respectively (Fig. 2). The AOC of the protein A-based process are 1.65-fold higher, which is mainly due to the very high costs of the protein A resin (US$16,250 per liter) that accounts for 79% of the total AOC. On the contrary, in the APTE process, the raw materials are the major contributors corresponding to 58% of the total AOC (Fig. 2A). A 4.5-fold higher raw material consumption per kg mAb is, indeed, observed for the ATPE process, consequently, leading to about 10- and 25-fold higher raw materials and waste treatment and disposal costs, respectively.

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WFI (water for injection) is the most consumed raw material for both processes representing the major contributor to the raw materials costs in case of ProA (84%) and the second major in case of ATPE (28%). In the ATPE-based capture process, PEG 3350 is the main contributor to the plant raw materials costs (53%) due to the large amount required per year.

According to this study, the ATPE process was shown to be considerably advantageous in terms of process economics, especially when processing high titer cell culture supernatants. In fact, this alternative process is able to purify continuously the same amount of mAbs reducing the annual operating costs from 14.4 to 8.5 million (US$/kg), which represents a reduction of 39%, when cell culture supernatants with mAb titers higher than 2.5 g/L are processed [1].

References

Combining microwave resonance technology with multivariate data analysis as a PAT/QbD approach to improve process understanding in pharmaceutical processes

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The pharmaceutical industry is encouraged within Quality by Design (QbD) to apply science-based manufacturing principles to assure quality not even of new but also of existing processes. We have developed a general strategy based on QbD principles to be applied to existing industrial pharmaceuticals fluid bed granulation processes. The three steps involved are: 1) implementation of Process Analytical Technology (PAT) monitoring tools at the industrial scale process, combined with multivariate data analysis (MVDA) of process and PAT data to increase the process knowledge; 2) execution of scaled-down designed experiments at a pilot-scale, with adequate PAT monitoring tools, to investigate the process response to intended changes in Critical Process Parameters (CPPs); and finally 3) the definition of a process Design Space linking CPPs to Critical to Quality Attributes (CQAs), within which product quality is ensured by design, and after scale-up enabling its use at the industrial process scale.

The proposed strategy was tested in an existing industrial process. Through enhanced process knowledge established a significant reduction of product CQAs variability already within quality specifications ranges was achieved by a better choice of CPPs values. The results of such step-wise development and implementation are described.

The novel PAT monitoring tools included a microwave resonance probe to measure in-situ real-time the granules density, moisture and temperature and a spatial filter velocimetry (SFV) probe to measure real-time the particle size distribution of the granules population (Fig. 1).

Acquiring data over a year of manufacturing batches using the on-line system of Fig. 1, and applying PCA (principal component analysis) to the multivariate signal obtain from such instrument, it was found that process performance varied significantly and showed seasonality effects (Fig. 2).

A scale-down campaign of several designed experiments (Fig. 3) examining the factors with the greatest impact on process performance (obtained via a preliminary risk-assessment to the industrial process), over the period of a year, enhanced process knowledge and lead to the proposal of an improved set of process conditions (Fig. 4) [2-4].

Under the new set of process conditions granules properties were optimized and the process is now consistently operated at a much higher and stable

Figure 1. The in-line probes used: microwave resonance probe (left) and spatial filter velocimetry (right).
throughput (Fig. 4). The full account has been described in the already concluded PhD dissertation [5].

References:


Figure 2. Multivariate process trajectories obtained from PCA of granules density, temperature and moisture time profiles. Profiles in blue relate to batches produced in colder winter/spring months, while profiles in red to warmer summer/autumn months [2-4].

Figure 3. Outline of the screening DOE campaign carried out at the industrial pilot scale (1:10).

Figure 4. Designed granulations have similar yields compared to nominal industrial ones and the tablets manufactured with the designed granules are in specification. Granules properties such as flowability (FTO, flow through an orifice) show much higher consistency.
G-protein coupled receptors (GPCRs) are a large class of ubiquitous receptors expressed in eukaryotic cells. Signaling molecules like hormones, neurotransmitters and small peptides can bind to GPCRs thus regulating a variety of cell functions ranging from gene expression levels to cell shape and function. Consequently, these receptors play a major role on the pharmaceutical industry. Indeed, 30% of the current market drugs are GPCRs targets. Still, new approaches for the identification of novel modulators are being developed not only to access new functions but also to increase throughput and thus accelerate drug discovery. These issues are being addressed at BERG in collaboration with INESC-MN (J.P. Conde, V. Chu) by integrating microfluidic technology and silicon photodiodes. The major goal is to develop miniaturized devices capable of monitoring GPCR activation in living cells.

GPCR screening assays rely on the recording of the average signal from thousands of cells upon addition of a candidate drug target. Typically, changes in the intracellular levels of key elements in the signaling cascade are monitored using fluorescence read-out systems such as microscopy or CCD cameras [1]. Major challenges of the inherent miniaturization process, is the scaling-down of the optical apparatus. Photodiodes are characterized by presenting high photosensitivity, low dark current and high frequency response. In particular, thin film photodiodes based on hydrogenated amorphous silicon (a-Si:H) are readily compatible with microfabrication techniques and consequently easily integrated "On-Chip" for acquisition of the optical signal.

For proof-of-concept studies, HEK 293T cell lines, endogenously expressing the Muscarinic M1GPCR was the chosen biological model. Activation of the M1 receptor can be easily monitored by following the rise in intracellular calcium (iCa²⁺) upon addition agonist. Typically, cells are stained with calcium sensitive fluorophores that exhibit enhanced fluorescence upon calcium binding. A positive signal is characterized by a steep rise in cell’s fluorescence, followed by a slow decay as desensitization of the GPCR occurs and calcium levels are restored to basal values (Fig 1A).

The intensity of the maximum signal can be correlated with different agonist concentrations in order to generate a dose response curve (Fig. 1B), thus enabling the calculation of the EC50 i.e. the concentration of agonist that elicits 50% of the maximum signal, which, in turn, represents a measure
The a-Si:H p-i-n photodiodes consist of a mesa junction obtained by sequentially depositing layers of 200 Å n+-a-Si:H, 5000 Å intrinsic a-Si:H and 200 Å p+-a-Si:H. However, the use of photodiodes for fluorescence measurements rely on the availability of adequate filters that cut the excitation light while allowing the transmission of the emission light. This was accomplished by depositing a 2 µm layer of amorphous silicon carbon-alloy (a-SiC:H)[2]. Figure 2A represents a schematic cross section of the device. Figure 2B represents the ratio of transmitted light of the a-SiC:H filter, showing a two order magnitude increase in transmittance of the filter on the emission wavelength when compared to the transmitted light at 490 nm (λex, Fluo4).

For the monitoring of GPCR activation, HEK 293 Cells (50 µl) were cultured overnight on PDMS wells, previously coated with fibronectin, followed by an incubation with Fluo4. After performing all the electronic connections, the cell-containing wells were placed on top of the photodiode and stimulated with 1 mM carbachol. Figure 3 shows the current density, J, obtained for a different set of experiments. An increase in J is observed when carbachol is added to the cell-containing wells.

Conclusions
The monitoring of GPCR M1 receptor activation was confirmed using a-Si:H photodiodes with integrated optical filters. On-going work is focusing on the miniaturization of both the cell and optical apparatus.

Acknowledgments BERG/NABL acknowledges Prof. João P. Conde, Virginia Chu and INESC-MN group for the fabrication of the photodiodes.

Rational engineering of *E. coli* strains for improved manufacturing of plasmid biopharmaceuticals


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Plasmid DNA (pDNA) biopharmaceuticals are being developed for veterinary and human applications in gene therapy and vaccination. Although significant advances have been made in plasmid design and downstream processing, the need to improve fermentation processes and pDNA production strains remains largely unmet. One of the key challenges is the achievement of a high-yield and cost-effective manufacturing process. The focus of this project, which is being developed in collaboration with the Prather Research Group at MIT, in the context of the MIT-Portugal Program, is to create improved pDNA production strains.

The gram-negative bacteria *Escherichia coli* is a well-studied and largely explored microorganism in the industry, and is the most common host for the propagation of pDNA. Nevertheless, most of the strains of *E. coli* used for pDNA production were created through a series of mutations to facilitate cloning of heterologous genes and production of recombinant proteins. Recently, new pDNA production strains were developed in order to obtain high pDNA yields. However, the new mutations were usually done in strains with highly mutagenized genetic background, such as DH5α, and it is not known if the strain genetic background would impact the effect of a new gene knockout or overexpression (Fig. 1) [1].

Genes in the central carbon metabolism are obvious targets for the engineering of high-yield pDNA strains, since the manipulation of such genes could enhance nucleotide synthesis, increase production of energy and reducing power, and minimize acetate formation. On the other hand, genes related to improving pDNA quality have also been common targets, as have genes that are involved in various other cellular processes relevant to pDNA production such as the stringent response and DNA replication [1].

To enhance the production of nucleotides and reduce organic acids synthesis, key genes on the glycolytic pathway were knocked out. The impact of host strain genetic background was investigated as well as the carbon source effect on pDNA production. Genes in the glycolytic pathway, which had been already proved to increase pDNA yields, such as *pykF* and *pykA* genes [2-3],

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**Figure 1.** *E. coli* K12 and derivatives - creation of new strains and relationship between different strains. (A) Lineage of MG1655 and W3110, close relatives of wild-type *E. coli* K12 [66]. (B) Generation of strains containing multiple mutations from MC1061, DH1 and JM101 [67-68]. Dark boxes represent commonly-used *E. coli* strains for plasmid DNA production and recent developments in *E. coli* strains designed for high yield pDNA processes. Full line arrows represent the relationship between the strains and dashed line arrows represent mutations carried from one strain to the other.
were first deleted in two different strains, MG1655ΔendAΔrecA (wild-type genetic background) and DH5α (highly mutagenized genetic background).

The deletion of endA and recA were also done in MG1655 in order to minimize recombination and non-specific digestion of DNA [4-5]. We observed that host strain genetic background impacts the effect of a specific gene knockout, since the double mutation pykF and pykA was efficient only in MG1655ΔendAΔrecA and not in DH5α. Plasmid DNA yields were higher in glycerol than glucose for the wild-type strain MG1655ΔendAΔrecA. However, all the strains containing mutation in the glycolytic pathway were more efficient in glucose [6].

Finally, we created a new pDNA production strain, starting from the wild-type MG1655ΔendAΔrecA, with the introduction of the knockout of pgi gene, to completely redirect the carbon flow into the pentose phosphate pathway (PPP). This strategy enhances NADPH formation and nucleotide synthesis, which were demonstrated to favor pDNA production (fig. 2). For the first time, a pgi mutant strain, GALG20 (MG1655ΔendAΔrecAΔpgi), was identified as a potential high-yield pDNA production strain. GALG20 produced 25-fold more pDNA (mg/g DCW) than MG1655ΔendAΔrecA in high concentration of glucose. The top-three strains in terms of high-yield pDNA production are identified in table 1 [6]. The main achievement of this study was thus the creation of high-yield pDNA production strains from the wild-type strain MG1655.

References

Table 1. Top three high-yield pDNA production strains identified versus a common strain for pDNA production, DH5α

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carbon source</th>
<th>Volumetric yield (mg/L)</th>
<th>Specific yield (mg/g DCW)</th>
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<tbody>
<tr>
<td>GALG20 (MG1655ΔendAΔrecAΔpgi)</td>
<td>Glucose</td>
<td>140.80 ± 0.76</td>
<td>19.08 ± 1.52</td>
</tr>
<tr>
<td>GALG11 (MG1655ΔendAΔrecAΔpykA)</td>
<td>Glucose</td>
<td>94.10 ± 2.74</td>
<td>13.05 ± 0.20</td>
</tr>
<tr>
<td>MG1655ΔendAΔrecA</td>
<td>Glycerol</td>
<td>79.31 ± 1.39</td>
<td>11.15 ± 0.48</td>
</tr>
<tr>
<td>DH5α</td>
<td>Glycerol</td>
<td>34.68 ± 0.63</td>
<td>4.40 ± 0.29</td>
</tr>
</tbody>
</table>

Strains were grown in shake flasks at 37°C. Standard error of mean (SEM) is shown in parentheses.
Embryonic stem cells (ESC) are undifferentiated cells that have the ability to either self-renew, giving rise to two identical pluripotent “daughter” cells, or to differentiate, producing specialized cells. These properties make them a very attractive cell source for stem cell-based therapies, for developmental biology studies and also for drug/toxicity-screening. Nonetheless, the successful implementation of stem cell-based technologies will require the ability to generate high numbers of cells with well-defined characteristics. For those reasons, the goal at the BERG-SCBL is to develop efficient scale-up strategies from the commonly used static culture systems (e.g. tissue culture plates) to dynamic culture systems such as stirred tank reactors (STR) operating under a continuous perfusion mode with cell retention. One important parameter in perfused cultures is the flow rate at which medium is renewed. The concentration of growth factors and nutrients is usually a growth-rate-limiting factor, as well as unfavorable pH, accumulation of inhibitory metabolites or a combination of some of these factors. An excessive medium exchange and/or an unnecessarily high perfusion rate would result in wasting these valuable components and overdilution of autocrine factors promoters of cell growth. The aim of this work was to study the influence of the residence time on the expansion of mouse ESC (mESC) using serum-free (SF) medium in a STR operating under a continuous perfusion mode with cell retention.

**Influence of the residence time on mESC proliferation**

A microcarrier-based STR system (Figure 1a), under SF conditions, was previously established for mESC expansion using a feeding strategy of 50% medium exchange every day (Fernandes-Platzgummer, 2011). Herein the residence times under continuous operation studied were 12, 24, 32, 48 and 96 hours. The residence time is defined as the time that the medium is exposed to the cell culture. The influence of the residence time on the expansion of mESC was studied in a perfused bioreactor culture system using SF medium. The agitation rate was set to 60 rpm. Values are represented as mean ± SEM.

Figure 1. Expansion of mESC in a stirred tank reactor. (a) New Brunswick Bioreactor (1.3L) and controller, (b) Effect of the residence time of culture medium on 46C mESC growth on Cultispher S microcarriers in a perfused bioreactor culture system using SF medium. Residence times of 96h (p, n=1), 48h (■, n=3), 32h (●, n=2), 24h (r, n=2) and 12h (○, n=2) were studied. Growth curve of the cells fed once per day with 50% medium change every 24 hours (□, n=3) is also depicted in figure 1. Cells were inoculated at 5×10⁴ cells/mL on 1 mg of microcarriers per mL of culture medium and agitation rate was set to 60 rpm. Values are represented as mean ± SEM.
32, 48 and 96 hours and the respective growth curves of mESC are represented in Figure 1b, in comparison to the previously established discontinuous feeding strategy. The operational parameters were set to: temperature 37°C, pH 7.2, agitation rate 60 rpm, airflow rate 100-200 ccm and dissolved oxygen concentration 20%.

As it can be seen in Figure 1b, with the exception of 96h, all the residence times supported mESC expansion. For the residence time of 96h, in which only 25% of the medium was exchanged per day, the cells stopped growing after day 7 probably due to nutrients depletion and accumulation of inhibitory metabolites. For the other four residence times studied, growth curves followed the expected pattern leading to the maximum cell numbers and specific growth rates presented in Table 1. Comparing the growth curve of the cells expanded with a culture medium residence time of 48h (i.e. 50% medium renewal/day) and the growth curve of the cells fed once per day (i.e. 50% medium renewal/change), it can be observed that shifting the feeding scheme from discontinuous to continuous mode increased the cell density by 2-fold. An explanation could be that in the discontinuous medium exchange protocol, a large portion of medium is replaced at a time (50% every 24h) which might affect cell growth in two ways: if medium exchange is performed too early in culture or a large portion of medium is replaced at a time, a dilution of important autocrine factors can occur; if medium exchange is performed too late, an accumulation of toxic metabolic byproducts can inhibit cell growth and ultimately lead to cell death. On the other hand, in the continuous perfusion mode, the addition and removal of metabolites and other inhibitors can be made in a controlled way without the dilution of the autocrine factors necessary to mESC expansion. Permanent medium exchange via perfusion is thus an important step forward in the automation and standardization of the culture conditions.

To evaluate which residence time would contribute to a higher cell yield, the maximum total cell number achieved was divided by the total volume of medium used. As it can be seen in Table 1, the maximum cell yields, $1.4x10^6$ and $1.2x10^6$ cells per mL of medium used, were attained with the residence times of 48 hours and 32 hours, respectively.

Importantly, mESC expanded in the fully controlled STR, using SF medium, retained the expression of pluripotency markers and their differentiation potential into cells of the three embryonic germ layers (ectoderm, mesoderm and endoderm).

The controlled bioprocess developed herein is potentially adaptable to other cell types, including human ESC and induced pluripotent stem cells, thus representing a promising starting point for the development of novel technologies for the controlled production of differentiated derivatives from human pluripotent stem cells.

References:

### Table 1. Growth kinetic characterization and cell yields for the different residence times.

<table>
<thead>
<tr>
<th>Residence times</th>
<th>12 hours</th>
<th>24 hours</th>
<th>32 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum cell number (cells)</td>
<td>4.0x10^6</td>
<td>5.5x10^6</td>
<td>6.4x10^6</td>
<td>5.5x10^6</td>
</tr>
<tr>
<td>Fold increase</td>
<td>114±5</td>
<td>156±10</td>
<td>184±8</td>
<td>156±19</td>
</tr>
<tr>
<td>Specific growth rate (day⁻¹)</td>
<td>1.3±0.1</td>
<td>1.6±0.2</td>
<td>1.3±0.2</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>Doubling time (day)</td>
<td>0.6±0.2</td>
<td>0.4±0.1</td>
<td>0.5±0.1</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>Cell Yield (Cells/mL of medium used)</td>
<td>0.4x10^6</td>
<td>0.8x10^6</td>
<td>1.2x10^6</td>
<td>1.4x10^6</td>
</tr>
</tbody>
</table>
Multifactorial analysis of embryonic stem cell self-renewal reveals a crucial role of GSK-3-mediated signaling under hypoxia

H.S.C. Barbosa, T.G. Fernandes, T.P. Dias, M.M. Diogo and J.M.S. Cabral

Work previously performed in our group showed that culturing mouse embryonic stem (mES) cells under different oxygen tensions gave rise to different cell proliferation patterns and commitment stages depending on which signaling pathways are activated or inhibited to support mES cell self-renewal [1]. These findings indicate that mES cell self-renewal and pluripotency, which are dependent on multifactorial signaling networks, can be influenced by different oxygen levels. However, the molecular mechanisms that regulate stem cell fate and function under these conditions are not well understood.

Multifactorial Analysis of Signaling Networks at Different Oxygen Tensions

To elucidate and dissect how each signaling pathway is functioning at physiological and non-physiological oxygen tensions, we have used a multifactorial approach and response surface methodology. The effects of three independent variables LIF, CHIR99021 (CHIR) and PD0325901 (PD) on the specific growth rate (SGR) and the efficiency in colony formation of mES cells were determined using a face-centered composite design (FC-CD) approach. Therefore, the sole and interactive influence of MEK/ERK pathway inhibition, activation of Wnt/β-Catenin by GSK-3 inhibition, and activation of LIF/STAT3 signaling, was statistically evaluated during expansion of mES cells at different oxygen tensions (Fig. 1). The obtained models were then validated to confirm the effects of each signaling molecules in mES cell expansion and pluripotency at different oxygen tensions.

Effect of Hypoxia on Mouse ES Cell Expansion

According to the models described above, it is possible to observe that generally mES cells significantly reduce its propagation in serum-free medium at physiological oxygen levels as compared to 20% oxygen conditions (Fig. 2). Taking the highest specific growth rate conditions in both oxygen levels this represents a 6.7 reduction in the cumulative fold increase in total cell number at the end of the tenth day as compared to normoxic (20%) oxygen levels. This higher mES cell proliferation rate in normoxia was obtained when the culture medium was supplemented with only one of the three factors: LIF. None of the two small molecule inhibitors (PD and CHIR) had a significant impact on mES cell expansion at this O₂ level, indicating that LIF/STAT3 signaling was dominant over MEK/ERK and Wnt/β-Catenin signaling pathways (Fig. 2a).

On the contrary, under hypoxia, the activation of the Wnt/β-Catenin signaling via inhibition of GSK-3β had a significant influence over the mES cell expansion. In hypoxia, the culture conditions that maximize mES cell specific growth rate are obtained when LIF is supplemented into culture medium at a concentration of 720 U/mL and CHIR at approximately 3 μM (Fig. 2b). These observations indicate that Wnt signaling mediated by the canonical pathway is not absolutely sufficient and requires an synergistic action of LIF to maintain self-renewal of mouse ES cells under low oxygen levels.

Effect of Hypoxia on Mouse ES Cell Pluripotency

We also evaluated the capacity of different culture conditions to support mES cell pluripotency by growing these cells at clonal densities. High efficiencies of colony formation indicate that mES...
cells have a high percentage of survival and can generate a high number of colonies, suggesting that the culture conditions employed can better support mES cell pluripotency. Culturing mES cells at low density in 20% O₂ tensions resulted in the formation of a high number of colonies, indicating that the culture conditions employed could better support mES cell pluripotency. On the other hand, 2% O₂ tensions, absence of CHIR resulted in a significant decrease in cloning efficiency, while the presence of CHIR and LIF are essential for high colony formation efficiency at low oxygen tensions under low cell densities. This was further validated by quantitative PCR and immunofluorescence staining of specific pluripotency markers. The expression of pluripotency genes was up-regulated when CHIR or the two inhibitors were added with LIF to the culture medium at 20% O₂. On the other hand, at 2% O₂, absence of CHIR resulted in down-regulation of pluripotency markers, while presence of this molecule in combination with LIF causes maintenance or increasing expression of core pluripotency genes, confirming the results predicted by our models. Furthermore, at 2% O₂ the absence of CHIR resulted in colonies with differentiated morphology and cytoplasmic localization of Nanog protein, an indication of early commitment of these cells. Collectively, this approach provided new insights into the mechanisms by which oxygen influences mES cell self-renewal and pluripotency while distinct pathways are activated or inhibited. This modeling approach revealed that at lower O₂ tensions, LIF/STAT3 signaling and Wnt/β-Catenin, in particular, show a significant role towards maintenance of mES cell self-renewal and pluripotency. Our results add new insights into the mechanisms by which oxygen tension influences mES cell fate, and GSK-3 inhibition in particular showed an important role towards maintenance of ES cell pluripotency.

References

Scientific Output

2011
BERG Annual Report
Publications

Articles in Peer-reviewed Journals


Coutinho, C.P., de Carvalho, C.C.C.R., Madeira, A., Pinto-de-Oliveira, A., Sá-Correia, I., "Burkholderia cenocepacia clonal phenotypic variation during three and a half years of residence in the lungs of a cystic fibrosis patient", Infection and Immunity, 79(7), 2950-2960


Marques, M.P.C., Fernandes, P., “Microfluidic devices: Useful tools for bioprocess intensification”, Molecules, 16(10), 8368-8401


Tyagi, M., da Fonseca, M.M.R., de Carvalho, C.C.C.R., "Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes", Biodegradation, 22(2), 231-241

Biotecnol. J., 6, 362-363 (Editorial accompanying article from Oliveira et al., 2011, 6, 378-391)

Articles in National Journals


Prazeres, D.M.F., "Biomimica", Ingenium, Jan/Fev, 86-88


Articles in Conference Proceedings


Editorials about BERG publications


Books


Book Chapters


Lourenço, N.M.T., Nunes, A.V.M., Duarte, C.M.M., Vidinha, P., "Ionic liquids gelation with polymeric materials: the ion jelly approach" in: Applications of Ionic Liquids in Science and Technology (Scott Handy editor), Middle Tennessee State University, USA, pp 155-172


Patents

de Carvalho, C.C.C.R., Marques, M.P.C., "Dispositivo para diluições sucessivas em microplacas", Provisional patent nr 105887. Priority date: 14 September 2011

Invited Oral Communication

International Conferences

Hakemeyer, C., Werz, S., Folque, F., José, G., Menezes, J.C., Strauss, U., “At-Line NIR spectroscopy as a simple and effective PAT monitoring technique in mab cultivations during process development and manufacturing”, AIChE Annual Meeting, Minneapolis, USA, October


Lourengo, V., Herdling, T., Reich, G., Menezes, J.C., Schewitz, J., “Combining microwave resonance technology to multivariate data analysis as a novel PAT tool to improve process understanding in fluid bed granulation”, AIChE Annual Meeting, Minneapolis, USA, October

Menezes, J.C., “From process-centered to product-centered QbD”, EUROPACT 2011, Glasgow, Scotland, April [Keynote Lecture]

Menezes, J.C., “Quality by design: Tools and Platforms”, 5th International Congress Pharma. Engineering, Graz, Austria, September

Menezes, J.C., “PAT in different industries: Challenges & Opportunities for NIRS”, NIR2011, Cape Town, South Africa, May [Keynote Lecture]

Menezes, J.C., “Modern pharmaceutical development and manufacturing: A decade into using multivariate data analysis”, - 11th annual conference of the European Network for Business and Industrial Statistics | ENBIS-11, Coimbra, Portugal, September

Menezes, J.C., “Process Analytical Technology (PAT) across different industries: Challenges and
opportunities in Process Development", - The 11th International Chemical and Biological Engineering Conference | CHEMPOR 2011, Caparica, Portugal, September

Schewitz, J., Herdling, T., Lochmann, D., Reich, G., Menezes, J.C., "Real-Time release strategy in MERCK SERONO: A pharmaceutical industry perspective", 25th International Process Analytical Technology Forum | IFPAC, Baltimore, Maryland (USA), January

National Conferences

Fernandes, P. "Miniaturization in bioprocesses: a resilient approach or just another fad?" 4th Joint National Congress of Microbiology and Biotechnology | Microbiotec11, Braga, Portugal, December

Madeira, C., Cabral, J.M.S., "Células estaminais e cartilagem", 3º Curso teórico-prático de Cartilagem Articular, Lisbon, Portugal, November

Madeira, C., Cabral, J.M.S., "Gene delivery to adult stem cells: pre-clinical studies and clinical trials", IX Encontro de Engenharia Biomédica, IST/FMUL, Hospital de Santa Maria, Lisbon, Portugal, November

Oliveira, P.H., "Biofármacos: Desafios e Limitações", Tertúlias FNACiência, FNAC Guimarães, Portugal, June

Oliveira, P.H., "Da sala de aula ao laboratório – Uma experiência na primeira pessoa", Jornadas de Engenharia Química e Biológica (JEQB), Instituto Superior Técnico, Lisbon, Portugal, March

Oral Communications

International Conferences


de Carvalho, C.C.C.R., "Bacterial adaptation to anti-neoplastic agents involve biofilm formation", 15th International Biodeterioration & Biodegradation Symposium | IBBS-15, Wien, Austria, September


Raiado-Pereira, L., Carapeto, A., Mateus, M., Botelho-do-Rego, A.M., "Grafting hydrophobic and affinity interaction ligands on membrane adsorbers: a close-up view by X-ray Photoelectron Spectroscopy", 11th International Chemical and Biological Engineering Conference | CHEMPOR 2011, Lisbon, Portugal, September


Nunes, M.A., Fernandes, P.C., Ribeiro, M.H., "Microliter plates as a representative system for enzymatic hydrolysis with PVA-lens shaped particles", 19th Biennial Meeting of the International So-


National Conferences


Barbosa, H.S.C., Fernandes, T.G., Diogo, M.M., Cabral, J.M.S., “Application of a central composite design for modeling mouse embryonic stem cell self-renewal at different O2 levels”, 6th International Meeting of the Portuguese Society for Stem Cells and Cell Therapy | SPCE-TC, Cantanhede, Portugal, April


dos Santos, F., Lobato da Silva, C., Andrade, P.Z., Abecasis, M.M., Gimble, J.M., Campbell, A.M., Boucher, S., Roos, E., Kuligowski, S., Chase, L.G., Vemuri, M.C., Cabral, J.M.S., “Clinical grade expansion of human mesenchymal stem cells using a microcarrier-based system under serum-free and xeno-free conditions”, 6th International Meeting of the Portuguese Society for Stem Cells and Cellular Therapy | SPCE-TC, Cantanhede, Portugal, April

Fernandes, T.G., Rodrigues, C.A.V., Miranda, C.C., Diogo, M.M., Cabral, J.M.S., “Towards fully defined culture systems for human induced pluripotent stem cell expansion” 4th Joint National Congress of Microbiology and Biotechnology | Microbiotec11, Braga, Portugal, December


during the *ex-vivo* expansion of human mesenchymal stem cells for clinical applications”, 6th International Meeting of the Portuguese Society for Stem Cells and Cellular Therapy | SPCE-TC, Cantanhede, Portugal, April

**Poster Communications**

**International Conferences**


de Carvalho, C.C.C.R., “Improving the bioremediation abilities of *Rhodococcus erythropolis*”, 15th International Biodeterioration & Biodegradation Symposium | IBBS-15, Wien, Austria, September


2011, Sicily, Italy, October


Oliveira, P.H., da Silva, C.L., Cabral, J.M.S., “Unusual DNA structures and instability motifs correlate with human mitochondrial deletion breakpoints involved in genetic disorders and cancer”, 11th International Symposium on Mutations in the Genome, Santorini, Greece, June


National Conferences

Barbosa, H.S.C., Fernandes, T.G., Dias, T.P., Diogo, M.M., Cabral, J.M.S., “A factorial design approach for modeling mouse embryonic stem cell self-renewal at different O2 levels”, 4th Joint National Congress of Microbiology and Biotechnology | Microbiotec11, Braga, Portugal, December


Coutinho, C.P., de Carvalho, C.C.C.R., Madeira, A., Pinto-de-Oliveira, A., Sá-Correia, I., “Burkholderia...
cenocepacia clonal phenotypic variation during long-term colonization of a cystic fibrosis patient lungs”, 4th Joint National Congress of Microbiology and Biotechnology | Microbiotec11, Braga, Portugal, December


**Dissertations**

**Ph.D. Thesis**


Ana Gabriela Gonçalves Neves Gomes, PhD in Biotechnology, “Intermediate recovery of plasmid DNA based on aqueous two-phase systems and phenyl-boronate adsorption”, UTILITYST (Supervisors: D.M.F. Prazeres, M.R. Aires-Barros)

Ana Margarida Pires Fernandes Platzgummer, PhD in Bioengineering, “Bioreactor culture systems for the expansion of mouse embryonic stem cells”, UTILITYST (Supervisors: J.M.S. Cabral, C. Lobato da Silva, M.M.R. Diogo)


Francisco Ferreira dos Santos, PhD in Biotechnology, “Isolation and ex-vivo expansion of mesenchymal stem cells for supplementation during hematopoietic stem cell transplantation”, UTL/IST (Supervisors: J.M.S. Cabral, C. Lobato da Silva)

Isabel Filipa Prates Acciaiol Hilário Ferreira, PhD in Bioengineering, “Biodesulfuration of crude oil by whole cells of Rhodococcus erythropolis”, UTL/IST (Supervisors: M.R. Aires Barros, C.C.C.R de Carvalho, D.I.C. Wang)

Pedro Miguel Zacarias Andrade, PhD in Bioengineering, “Novel approaches for the isolation and ex-vivo expansion of hematopoietic stem cells from human umbilical cord blood for cell therapy”, UTL/IST (Supervisors: J.M.S. Cabral, C. Lobato da Silva)

Vera Mónica de Campos Loures Lourenço, PhD in Chemical Engineering, “A quality by design study of an industrial fluid bed granulation process”, UTL/IST (Supervisors: J.C. Menezes, D. Lochmann)

**M.Sc. Thesis**

Ana Rita de Matos Parente Vasconcelos, MSc in Biological Engineering, “Concepção e desenvolvimento de um bloqueador de cimento ósseo”, UTL/IST (Supervisors: M.R. Aires Barros, L. Pinto)

Cátia M.M. Sousa, MSc in Pharmaceutical Engineering, “The Application of Quality by Design to Evaluate the Robustness of an Analytical Method”, UTL (Supervisors: J.C. Menezes, S. Queirós)

Cláudia Daniela Canelas Miranda, MSc in Biotechnology, “Towards fully defined culture systems for human induced pluripotent Stem Cell expansion”, UTL/IST (Supervisors: M.M. Diogo, T. Fernandes)

David Soares da Conceição, MSc in Bioengineering and Nanosystems, “Stem Cells in microfluidics - Controlling the cellular environment of microspotted Stem Cells”, UTL/IST (Supervisors: M.M. Diogo, J.P. Conde)

Daniel Filipe Camarneiro Silva, MSc in Biotechnology, “Antibody separation using aqueous two-phase systems in a microfluidic”, UTL/IST (Supervisors: M.R. Aires Barros, J.P. Conde)

Elisabete Marques Ribeiro, “Towards production scale with microreactors. Early steps to crack the paradox”, UTL/IST (Supervisor: P. Fernandes)
Filipa Esteves Leal Rodrigues de Carvalho, MSc in Biological Engineering, "Design of validation master plan for pharmaceutical industry and process validation of lyophilized drug", UTL/IST (Supervisors: J.M.C. Menezes, S. Pereira)

Filipa Fiel do Carmo Glórias Ferreira, MSc in Biotechnology, "Novel plasmid-based vectors for gene delivery to Neural Stem Cells", UTL/IST (Supervisor: C. Madeira)

Francisco Tavares Marinho Mendes, MSc in Biological Engineering, "Estudo da eficiência de um processo de produção de bolachas sustentado na gestão da qualidade", UTL/IST (Supervisors: M. Mateus, R. Machado)

Isabela Dodd Gueiros, MSc in Biotechnology, "Screening enzymatic systems for selective methyl ester production", UTL/IST (Supervisors: F.C. Ferreira, P. Fernandes, C. Fonseca)

Irina Neves Simões, MSc in Biotechnology, "Isolation, characterization and ex-vivo expansion of mesenchymal stem cells from umbilical cord matrix" (Supervisors: J.M.S. Cabral, C. Lobato da Silva)

Joana Baltazar Domingues, MSc in Biological Engineering, "Stability assessment of biopharmaceutical formulations", UTL/IST (Supervisors: A.M. Azevedo, J.A. Santos)

Joana da Costa Branco, MSc in Biological Engineering, "Development of a yeast based platform for the screening of compounds that modulate TTR toxicity", UTL/IST (Supervisors: F.C. Ferreira, P. Calado)

Joana Lopes Pereira, MSc in Biological Engineering, "Development of meat alternatives - Understanding fiber formation of vegetable proteins", UTL/IST (Supervisors: M. Mateus, F. van de Velde)

Joana Rita Pires Bentes Gil, MSc in Biotechnology, "Development of DNA vaccines prototypes against avian influenza viruses", UTL/IST, (Supervisors: G.A. Monteiro, M. Fevereiro)

José Frederico Silva Oliveira, MSc in Biological Engineering, "An integrated process for the purification of antibodies based on magnetic particles and aqueous two-phase systems", UTL/IST (Supervisors: M.R. Aires Barros, A.M. Azevedo)

João Miguel da Costa Medeiros, MSc in Biological Engineering, "Elucidation of endogenous haematopoietic cytokines production in a three-dimensional biomimicry of human bone marrow", (Supervisors: C. Lobato da Silva, A. Mantalaris)

João Pedro dos Santos Borges, MSc in Mechanical Engineering, "Desenvolvimento de técnicas baseadas em filmes de células bacterianas para aplicação em ensaios não destrutivos (END) de materiais de Engenharia", UNL/FCT (Supervisors: T. Santos, C.C.C.R. Carvalho)

João Porfírio da Silva Burgal, MSc in Biological Engineering, "Production of recombinant human cytochrome P450 (1A1) in E. coli JM109: Fed-batch fermentation in 20 L scale with a novel phage resistant strain", (Supervisors: D.M.F. Prazeres, M. Kitzelmann)

João Rodrigo Cardoso Trabuco, MSc in Biotechnology, "Towards the miniaturization of cell assays for GPCR monitoring", UTL/IST (Supervisors: D.M.F. Prazeres J.P. Conde)

Márcia Andreia Faria da Mata, MSc in Biological Engineering, "Tools for transient manipulation of HSC using non-integrating retroviral vectors", (Supervisors: C. Lobato da Silva, S. Howe)

Marina Eduarda Santos Valada Monteiro, MSc in Biotechnology, "Design of a liposome-based chromatographic membrane and its use for final plasmid DNA purification from Escherichia coli lysate contaminants", UTL/IST (Supervisor: M. Mateus)

Marta Taveira Santos Castro Silva, MSc in Cellular Biology, "Avaliação da capacidade de extractos voláteis de plantas aromáticas para inibir a formação de biofilmes bacterianos", FCUL (Supervisors: C.C.C.R. Carvalho, A.C. Figueiredo)

Nancy Hachicho, Master of Science, "Adaptation of Rhodococcus opacus to different chlorophenols and carbon sources", Universität Leipzig (Supervisors: H.J. Heipieper, C.C.C.R. Carvalho)

Nicolau F. Dehanov, MSc in Pharmaceutical Engineering, "Técnicas de calibragão espectroscópica baseadas na estimativa do ruído espectral e do sinal de resposta aplicadas a espectros de infravermelhos médios (MIR) de amostras de culturas de células estaminais", FF/UL (Supervisor: J.C. Menezes)
Núria Catarina Mendes da Silva Lopes, MSc in Biotechnology, “Purification of monoclonal antibodies by phenyl boronate chromatography”, UTL/IST (Supervisors: A.M. Azevedo, M.R. Aires Barros)

Pedro Almeida Nolasco, MSc in Bioengineering and Nanosystems, “Structural and mechanical characterization of Sialoliths, UTL/IST (Supervisor: P. Almeida de Carvalho, M.M. Diogo)

Raphaël Faustino Canadas, MSc in Bioengineering and Nanosystems, “Electrospun nanofibers for human stem cell cultivation”, UTL/IST (Supervisors: F.C. Ferreira, C. Lobato da Silva)

Roksana Maria Pirzgalska, MSc in Biotechnology, “Optimization of aqueous two-phase systems for human hematopoietic stem cells separation”, UTL/IST (Supervisors: M.R. Aires Barros, A.M. Azevedo)

Sandra Cristina Sarmento Donato dos Santos e Silva, MSc in Biotechnology, "Dielectrophoresis - A Biological Approach - Positive and Negative Dielectrophoresis of E. coli in a Microfluidic Environment", UTL/IST(Supervisors: J.P. Conde, D.M.F. Prazeres)

Sofia Machado Pinheiro, MSc in Biochemistry, "Desenvolvimento de métodos para estudo de inibidores da acetilcolinesterase (Tratamento sintomático da Doença de Alzheimer)", UL/FC (Supervisors: M.L.M.O.M Serralheiro, P. Fernandes)

Sofia de Oliveira Dias Duarte, MSc in Applied Microbiology, "The role of calcium in Saccharomyces sp. In response to etanol stress", FCUL, (Supervisors: G.A. Monteiro, A. Tenreiro)

Tatiana Vieira Arriaga, MSc in Biological Engineering, "Controlled and tailored denaturation and aggregation of whey proteins", UTL/IST (Supervisors: M. Mateus, T. Huppertz)

Teresa Margarida da Silveira e Silva Galhoz, MSc in Biotechnology, “Production of monoclonal antibodies by hybridoma cell culture”, UTL/IST (Supervisors: A.M. Azevedo, C. Lobato da Silva)


Tomás Miguel de Freitas Dias, MSc in Bioengineering and Nanosystems, "Magnetoresistive Chip-based platform for the evaluation of cfDNA integrity as a potential biomarker in cancer diagnosis", UTL/IST (Supervisor: G.A. Monteiro) 

Vera Sequeira Ribeiro Guerra, MSc in Biotechnology, "High throughput in biocatalysis: steroid bio-conversions", UTL/IST (Supervisors: P. Fernandes, M.P.C. Marques)

Awards

UTL/Santander Totta Scientific Award
Pedro Fernandes was distinguished with the UTL/Santander Totta Scientific Award, in the area of Biological Engineering.

Carla C.C.R. de Carvalho was distinguished with an Honorable Mention by UTL/Santander Totta, in the area of Biological Engineering.

UTL/Deloitte Young Researchers Award
Nuno M.T. Lourenço has been distinguished with the Young Researchers UTL/Deloitte Award in the scientific areas of Chemistry and Biochemistry.

Filipa Ferreira has been distinguished with the Young Researchers UTL/Deloitte Award in the scientific areas of Biological Engineering and Biotechnology.

Roche Young Investigator Award
Luis Borlido was distinguished with the Roche Younger Investigator Award 2011 for best oral communication, at the Affinity 2011, the 19th biennial meeting of the International Society for Molecular Recognition, Tavira, Portugal.

João Trabuco was distinguished with the Roche Younger Investigator Award 2011 for best oral communication, at the Affinity 2011, the 19th biennial meeting of the International Society for Molecular Recognition, Tavira, Portugal.
Oral Presentation Award

I. Filipa Ferreira was distinguished with the Best Oral Presentation Award for the presentation: I.Filipa Ferreira, Carla C.C.R. de Carvalho, Daniel I.C. Wang, M. Raquel Aires-Barros, Crude oil microbial desulfurization: a viable green technology for sulfur elimination in refineries. ChemPor2011, Lisbon, 5-7 September 2011.

Poster Presentation Award

Marina Monteiro was distinguished with the Best Poster Presentation Prize in Bioprocess Engineering for the presentation: Monteiro, M.E., Raiado-Pereira, L., Mateus, M., Prazeres, D.M.F., "Design of a liposome-based chromatographic membrane and its use for final plasmid DNA purification from Escherichia coli lysate contaminants", 4th Joint National Congress of Microbiology and Biotechnology | Microbiotec11, Braga, Portugal, December

Tiago Dias was distinguished with the Best Poster Presentation Prize in Cell and Tissue Engineering and Biomaterials for the presentation: Barbosa, H.S.C., Fernandes, T.G., Dias, T.P., Diogo, M.M., Cabral, J.M.S., "A factorial design approach for modeling mouse embryonic stem cell self-renewal at different O2 levels", 4th Joint National Congress of Microbiology and Biotechnology | Microbiotec11, Braga, Portugal, December
## Staff

### Faculty
- Joaquim M.S. Cabral
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- Duarte Miguel Prazeres
- Luís Fonseca
- José Menezes
- Cláudia Lobato da Silva
- Gabriel Monteiro
- José Santos
- Maria Ângela Taipa
- Marília Mateus
- Frederico Ferreira

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- M. Margarida Diogo
- Pedro Fernandes
- Teresa Catarina Madeira

### PhD Students
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- Cláudia Miranda
- Cláudia O. Silva
- David Malta
- Filipe Carvalho
- Geisa Gonçalves
- Irina Simões
- Irina Pinheiro
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- Jonathan de la Vega
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- Luis Raiado Pereira
- Michaela Simcikova
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- Mónica Coelho
- Nuno Faria
- Patricia Soares
- Ricardo Figueiredo
- Rimenys Jr. Carvalho
- Salomé Magalhães
- Tiago Dias
- Tomás Dias
- Vera Lourenço

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- Carlos Rodrigues
- Dragana de Barros
- Francisco dos Santos
- Hélder Barbosa
- Marco Marques
- Nuno Lourenço
- Pedro Oliveira
- Pedro Andrade
- Sara Badenes
- Sofia Martins
- Tiago Fernandes

### Master Students
- Ana Rosa
- Ana Vencá
- Andreia Dias
- Andreia Fernandes
- Andreia Matos
- Antónia Pinto

### Research Assistants
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- Mário Fonseca
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- Sofia Duarte

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- Joana Batista
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- Marta de Castro Silva
- Marta Silva
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- Raquel Correia
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