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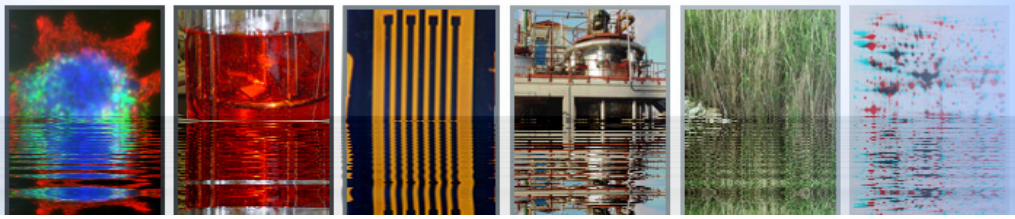


BOOK OF ABSTRACTS OF THE 2ND MEETING

Universidade do Minho

CAMPUS DE GUALTAR

BRAGA, 23-24 OCTOBER 2010

Book of Abstracts of the 2nd Meeting of the Institute for Biotechnology and Bioengineering

23-24 October 2010, Braga, Portugal

Publisher:

Universidade do Minho, Centro de Engenharia Biológica

Campus de Gualtar, 4700 Braga, Portugal

ISBN: 978-972-97810-7-0

This publication was sponsored by:

FCT Fundação para a Ciência e a Tecnologia

MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

Foreword

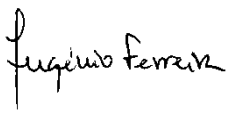
The “Laboratório Associado” Institute for Biotechnology and Bioengineering (IBB) is a research and development (R&D) unit, founded in October 2006 aiming to be a strategic infrastructure for the development of the Portuguese R&D and innovation policies in the areas of Biotechnology, Bioengineering, Biomaterials and Life, Biomedical and Agricultural Sciences. IBB combines its R&D activities with advanced education, technology transfer, consulting and services, with the aim of fostering the industrial, health, agriculture and environmental sectors.

IBB has a strategic and ambitious research plan to respond to the new the challenges, which have resulted from the extraordinary developments and breakthroughs in subjects like Molecular and Cell Biology, Genomics, Sysrems and Synthetic Biology, Biomaterials, Stem Cell and Tissue Engineering, or Nanotechnology. All these cutting-edge and fast growing/moving topics clearly demand for a mid/long term investment in both research infrastructures and human resources. The IBB contribution to these efforts relies on the integration of different scientific and technological subjects and competences of excellence, through the Centre for Biological and Chemical Engineering (Instituto Superior Técnico), the Centre for Biological Engineering (University of Minho), the 3B’s Research Group - Biomaterials, Biodegradables and Biomimetics (Universidade do Minho), the Centre of Genomics and Biotechnology (UTAD), the Centre for Molecular and Structural Biomedicine (Universidade do Algarve), and the Centre of Vegetal Biotechnology (Universidade de Lisboa).

IBB is already a major player in the Portuguese R&D related with biotechnology and bioengineering. Working as a research network in Portugal with strong international connections, IBB plays also a key role in the deployment of advanced doctoral programmes with close links to highly reputed institutions, aiming at contributing to the establishment of new start-up companies in the area of Biotechnology and Bioengineering.

During this meeting will be presented 12 research highlights, 79 oral presentations, and 97 posters in a total of 188 communications. This meeting is also characterized by providing significant amount of time dedicated to discussion within each thematic thrust area. We expect that such discussion, with the help of the international scientific advisory board, will identify strategic issues to foster IBB competitiveness both at the national and international levels.

Joaquim M.S. Cabral



Eugénio C. Ferreira

Manuel Mota

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Research Highlights

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Bioprocess Engineering and Biocatalysis: Highlights

Luis P. Fonseca and Raquel Aires-Barros

IBB-Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

Bioprocess Engineering and Biocatalysis, at the Bioengineering Research Group (BERG) from the Centre of Biological and Chemical Enginneering (CEBQ), aims to design and develop value-added bioproducts with potential application in the key areas of food and feed, aroma, pharmaceutical industry and biofuels. The current projects are focused on the development of technological platforms for biocatalysis and biomolecules purification organized in four major areas: i) Bioseparation, ii) Biocatalysis and Bioconversions, iii) Biosensors and Miniaturization, and iv) Bioenergy. The major achievements in Bioprocess Engineering include a novel separation process for the purification of monoclonal antibodies based on multistage liquid-liquid extraction in aqueous two-phase systems, which led to a joint world patent with Bayer Technology Services (Leverkusen, DE). A new strategy for penicilin G acylase immobilization in sol-gel was successfully developed, allowing a remarkable improvement of enzyme activity in comparison with previously reported sol-gel techniques; and the production of androstenedione from sitosterol was scaled-up from multi-well plates to bench-scale stirred bioreactor using suitable engineering parameters. The development of portable magneto-resistive biochips for pathogen microorganism with femtomolar DNA limit detection and using specific antibodies immobilized on nano-magneto particles (in collaboration with INESC-MN), is another successful project. Scientific projects currently going on will be present highlighting the work being developed in each of the four areas.

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Cross-cutting computational strategies to genome-scale modelling

Anália Lourenço, Sónia Carneiro, Isabel Rocha, Eugénio C. Ferreira

IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Hereby, the aim is to present some of our research efforts towards the reconstruction of genome-scale models. Namely, we focus on the development of cross-cutting computational strategies for the integration and validation of heterogeneous data in support to traditional manual curation and, describe application scenarios on the model organism *E. coli*.

We address the systematic comparison of database contents and the harvest and extraction of contents from scientific literature. Aiming to help researchers assess the gains and losses to be accounted for in biological repositories and thus, choose the most content-bearing repositories for each particular integration problem/domain, we have implemented a Web-alike report tool [1]. This tool analyses the contents of well-known repositories under user-specified integration scenarios considering the coverage of main biological entities (genes, proteins and compounds) and the evaluation of standard nomenclatures, common names and repository cross-links as elements of integration. Also, acknowledging that most biological data still lays on scientific literature and requires extensive and time-consuming manual curation, we have been developing literature screening and processing tools [2]. The goal is to systematise the search of relevant literature based on user-specified keywords and the extraction of relevant information by applying statistical approaches that exploit simple pattern matching, machine learning and ontological enrichment.

Considering the wide scope of current applications that can benefit from the analysis of large amounts of data, all our tools are publicly available through our group's Web pages (http://biopseg.deb.uminho.pt).

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Dendrimeric nanocarriers for intracellular cell- and tissue-engineering applications

Oliveira, J.M., Cerqueira, S.R., Salgado, A.J., Sousa, N., Mano, J.F., Reis, R.L.

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Cell- and tissue-engineering have benefited from the development of advanced strategies that can stimulate and control cells fate, *ex vivo* and *in vivo.* Intracellular and controlled delivery by means of using nanocarriers is gaining clinical significance as an alternative to traditional drug regimens. These have been designed not only to allow drug molecules or genetic material to be attached/loaded within the nanocarriers, but also to incorporate different functionalities for stimuli-responsiveness, and cellular and sub-cellular targetability/ traceability. We focused our attention in this fundamental problem, and have been proposing the use of surface engineered poly(amidoamine) dendrimers, the so-called carboxymethylchitosan/poly(amidoamine) dendrimer (CMCht/PAMAM) nanoparticles for intracellular drug delivery applications [1,2]. In this work, we report deeper studies on the physicochemical characteristics of these nanoparticles, namely the influence of incorporating different bioactive molecules such as dexamethasone and methylprednisolone. For traceability studies, fluorescein isothiocyanate (FITC) was linked to the CMCht/PAMAM dendrimer nanoparticles in order to investigate the uptake, mechanism of internalization and intracellular trafficking using rat bone marrow stromal cells. For targetability studies, CD11b antibody (microglia specific) was also bonded to the FITC-labelled CMCht/PAMAM dendrimer nanoparticles in order to render them a cell targeted profile. Cell viability was assessed through the ATP quantification and MTS assay. Immunocytochemistry was performed for the different glial cell types, so that the nanoparticles internalization could be assessed.

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Development of DNA vaccines against African trypanosomiasis encoding antigen-targeting sequences

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African trypanosomiasis (AT) is an emergent disease with significant social and economical impact. Since the available drug regimens are limited and highly toxic, the development of a vaccine would be extremely advantageous [1]. Ten DNA vaccine constructs were designed by linking the ISG75 and TSA genes from *Trypanosoma brucei* to DNA sequences that target the transport of the antigenic proteins to the MHC I and MHC II pathways [2]. The immunogenicity of ISG75 and TSA was demonstrated by the detection of antibodies against these proteins in infected mice. Cytokines with therapeutic interest were identified following immunisation with a number of DNA vaccine prototypes. Anti-ISG75 and anti-TSA antibodies were detected in vaccinated mice. The sequential administration of DNA vaccine encapsulated in chitosan-liposomes and ISG75 recombinant protein resulted in a 85-7,500-fold increase in antibody titres when compared with the DNA vaccine alone. Priming of the immune system and generation of memory response was elicited by immunisation with some plasmid DNA constructs. However, protective immunity was not observed. Histology changes induced by trypanosome antigens in popliteal lymph nodes sections were also analysed. T lymphocytes and macrophages were identified as the cells that primarily respond to infection. This work has contributed to the understanding of the immunobiology of AT and provides encouraging results for the development of a trypanosomiasis vaccine.

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The propagation of acoustic waves in thick shear mode devices to study biological processes – an integrative approach within IBB

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Acoustic wave sensors have been shown to be highly effective functional devices to monitor biomolecular binding in real time. The thickness shear mode (TSM) acoustic wave sensor is the most popular acoustic wave device, generally known as Quartz Crystal Microbalance (QCM). Upon real time monitoring the variation of the sensor resonance frequency and motional resistance, or dissipation factor, and using acoustic impedance spectroscopy and equivalent electrical circuit models, the acoustic wave signal can be cleared up to distinguish mass load from acoustic energy losses and charge induced parasite capacitive interferences. We have previously shown the effectiveness of this strategy in assessing both equilibrium and kinetic constants while simultaneously giving some incites regarding conformational alterations and hydrophobicity of adsorbed proteins at the sensor surface. We further extended this approach to study cell adhesion processes and DNA conformation. The rational is to find the variation of specific parameters and their physical association to the rigidity of the adsorbed layers as well as appropriate models of plasticity and viscoelasticity.

In this communication we present the physical basis of acoustic sensors and how they are or will be exploited to study stem cell adhesion and differentiation, and DNA transcriptor factors screening in two integrative research projects involving different groups within IBB.

Bioactive Self-assembling Matrices for Regenerative Medicine

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Hyaluronan (HA) is one of a group of polysaccharides typically found in the connective tissues of vertebrates and is one of the major ECM components in skin. The special physicochemical and biological properties of HA and its non-immunogenic nature have made this biopolymer a particularly useful substance for engineering bioactive matrices for cartilage and skin regeneration.

Self-assembling materials that can interact with cells and trigger the differentiation of human stem cells are of major interest in the field of regenerative medicine. Recently, a remarkable discovery was made on self-assembly of macromolecules and small molecules [1]. Under specific conditions, instant self-assembly between HA and peptide molecules of opposite charge, occurs at the liquid-liquid interface and can result in the formation of self-sealing sacs or 2D membranes. These matrices are mechanically robust (can even be sutured to tissues), are permeable to proteins, and can support cell viability and function. A major advantage of these systems is the ability to integrate directly in the structure biomolecular ligands for cell signalling.

Our research seeks to develop advanced biomaterial configurations that result from the self-assembly of HA and bioactive peptides. The vision is to develop, characterize and model the mechanical and biological properties of these self-assembling matrices. Currently, we are focused on the design and synthesis of peptide molecules incorporating self-assembling components and bioactive signalling sequences and explore the HA chemistry to drive the self assembly of the peptide structures. An array of advanced spectroscopy and microscopy techniques are being used for characterizing these self-assembling systems and we are testing how human cells respond to these artificial matrices. The findings of our research will be presented in this communication.

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Ecophysiology of long-chain fatty acids conversion to methane

D.Z. Sousa[1], A.J. Cavaleiro[1], M.A. Pereira[1], Salvador, A.F., J.I. Alves[1], H. Smidt[2], A.J.M. Stams[2], M.M. Alves[1]

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Syntrophic relationships are the key for biodegradation in methanogenic bioreactors. We review our work on the ecological and physiological features of syntrophic communities involved in the degradation of saturated and unsaturated long-chain fatty acids (LCFA). DGGE fingerprinting and sequencing showed the importance of *Syntrophomonas* closely related bacteria during batch and continuous degradation of unsaturated and saturated LCFA[1,2]. An obligatory syntrophic LCFA degrader – *Syntrophomonas zehnderi* – was isolated (in co-culture with *Methanobacterium formicicum*) from an oleate enrichment culture[3]. Oleate is an unsaturated LCFA with 18 carbon atoms and is one of the most common LCFA in wastewaters. The capability of degrading unsaturated LCFA is not widespread within *Syntrophomonas* genus. From the 11 *Syntrophomonas* species or subspecies that can use fatty acids only 3 are able to use oleate[4]. Enrichment cultures on oleate (unsaturated LCFA) and palmitate (saturated LCFA) resulted in distinct bacterial communities, which could be correlated with differences in the chain saturation[4]. Communities enriched on oleate could also degrade palmitate, but the opposite was not the case. The principle pathway of LCFA degradation is through β-oxidation, but the initial steps in the conversion of unsaturated LCFA are unclear. Currently, the genomes of *Syntrophomonas zehnderi* is being sequenced (DOE- Joint Genome Institute, http:www.jgi.doe.gov), and comparative genomics of this specie may shed more light on fatty-acid degrading pathways and the regulatory mechanisms that govern degradation of saturated and unsaturated fatty acids.

Acknowledgment: Financial support from Portuguese Science Foundation (FCT) and European Social Fund (ESF) through the PhD grants SFRH/BD/8726/2002 and SFRH/BD/24256/2005.

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ENVERG contribution to a cleaner and competitive environment

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Scale-up of constructed wetlands (CW) for the treatment of industrial wastewaters highly contaminated with organic aromatic molecules as well as for the nitrification and denitrification of nitrogenous compounds found in landfill leachates and other industrial effluents was successful. Besides landscape amenity these systems do not produce sludge as a by-product and mechanical equipment involved is scarce. Nonetheless, CW green status will only be accomplished if it is proved that the soil matrix is not a pollutant graveyard and that plants play an active role in the transformation of up taken pollutants beyond chemical stress answer by the self-defence mechanisms. So far, the classical approach through the characterization of plant tissues enzymatic array along with omics tools have demonstrated that *Phragmites sp.* adapt easily to the presence of a new molecule. Several organic aromatic molecules were transformed by *Phragmites sp.* leaves crude extract denoting the potential active role of CW vegetation. Research efforts involving phytotoxicity and physico-chemical characterization of soil in old full-scale systems led to promising preliminary results pointing towards a non-hazardous soil.

Waste to energy is also a currently active research area. Biological activity determination by the well-known respirometry tests is time consuming, not compatible with waste fuels production and was not useful to prevent fire risks or waste fuel biodegradability during storage. So, the development of a methodology to evaluate in a short time the biostability of waste fuels in which the biogenic content is enriched by waste fractions from municipal wastes mechanical and biological treatment units is being undertaken as a priority.

Studies on the efficacy of combined bioaugmentation and biostimulation treatments in soils contaminated with atrazine commercial formulations: The importance of ecotoxicological monitoring

C.P. Costa[1], S. Chelinho[2], M. Moreira-Santos[2], P. Viana[3], D. Lima[1], R. Ribeiro[2], A. M. Fialho[1], J.P. Sousa[2], C.A. Viegas[1]

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The *s*-triazine herbicide atrazine and its dealkylated metabolites are among the most frequently detected pesticides in soil as well as in water compartments due to runoff and leaching events. Concerns regarding their impact on human and ecosystems health have promoted the research on strategies to bioremediate environments contaminated with this herbicide. In the present work, bioremediation treatments involving bioaugmentation with the atrazine-mineralizing bacterium *Pseudomonas* sp. ADP and biostimulation with citrate proved to be effective for the removal of atrazine from soil microcosms comprising a crop soil from central Portugal spiked with atrazine commercial formulations [1, 2]. The formulations used contained atrazine as the single active ingredient [1, 2] or mixed with the chloroacetoanilide herbicide S-metolachlor. The doses tested were 10-, 20- and 200-fold higher than the recommended dose for an agricultural application, mimicking worst-case-scenarios (e.g. overuse, careless disposal, accidental spills) [1, 2]. To evaluate the efficacy of the application of the bioremediation treatments at the ecotoxicological level, soil samples and the respective eluates and leachates obtained from treated and non-treated soil microcosms, were tested for toxicity over standard soil and aquatic test species [2]. Experimental evidences will be presented that stress the importance of monitoring ecotoxicity, besides the fate of the herbicides and its metabolites in soil, during the implementation of the bioremediation processes, in order to assess the actual efficacy of the cleanup treatment and to get clues on its potential ecological impact.

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Nano structures for food applications

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Recently, nanotechnology emerged as one of the most promising and attractive research fields, with applications ranging from the aerospace to health industries. Food Industry can also benefit from nanotechnology applications – e.g. this technology offers the potential to improve bioavailability and solubility of different functional ingredients or multilayer films at the nano scale may improve transport properties of bioplastics.

The Pilot Plant Laboratory from CEB has been working on development different nanostructures with potential for food applications. Examples of such research are given in this presentation.

Nanoemulsions of β-carotene were prepared using a high-energy emulsification–evaporation technique. Process parameters such as time and shear rate of homogenization affected significantly particle size distribution in terms of volume-weighted mean diameter and surface-weighted mean diameter. Those nanoemulsions showed a good physical stability during 21 days storage. The stability was evaluated by the maintenance of size distribution. However, β -carotene retention inside the micelles and color were affected by storage.

As for nanolaminates, our research allowed characterizing the surface properties, water vapor permeability, and thermal and mechanical properties of a nanolayered film. The film was produced using two polysaccharides with opposite charges, chitosan and sodium alginate deposited on to aminolyzed/charged PET. Contact angle measurements showed differences in the films with a successively higher number of layers. SEM images allowed the measurement of the thickness of the layers. The properties of the obtained nanolayered film were significantly different from the ones measured in aminolyzed/charged PET, showing potential for application of such structures.

Satellite DNA: Patterns of Chromosome Evolution and Genome Remodelling

Sandra Louzada, Filomena Adega, Ana Paço, Sara Santos, Ana Vieira-da-Silva, Ana Borges, Susana Meles, Henrique Guedes-Pinto, Raquel Chaves

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Heterochromatic regions harbour satellite DNA sequences which are a very dynamic component of mammalian genomes, constituting an important factor of genomic plasticity. Recent research provide a large growing body of evidence indicating that tandem repetitive sequences and satellite DNA play an important role in mammalian evolution by promoting chromosomal rearrangements. Different satellite sequences co-exist in the genome, forming a satellite DNA library made of independent evolutionary units ruled by the mechanisms of concerted evolution, leading to the emergence of species-specific satellite profiles. There are several conjectures portrayed to explain the active role of satellite DNA in genomes remodeling. Since the breakpoints occur in the repetitive DNA blocks, the chromosome rearrangements would have low effects on the euchromatic genome by keeping syntenic segments intact. However, further experiments are needed to depict conclusive evidences about the causal involvement of satellite DNA in chromosomal evolution and will certainly allow the ascertainment of the mechanisms that effectively explain the role of satellite DNA in chromosome and in genome evolution.

Here, special emphasis will be given to the “hallmarks” that constitute true evidences of the involvement of heterochromatic regions and satellite DNA in the evolution of chromosomes and in genomes’ remodelling. The value of satellite DNA markers in the reconstruction of group phylogenies, models for chromosome rearrangement will be highlighted with examples from various mammalian groups such as Cetartiodactyla, Rodentia and Carnivora.

Portuguese aromatic flora: chemical *versus* molecular diversity and biological activity

Pedro, L.G.

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The Portuguese Flora is particularly rich in aromatic and medicinal species, some of them endemics. The knowledge on plant species with potential economical interest can contribute for a sustainable exploitation of the Portuguese Flora.

The Plant Biotechnology Research Group has been involved on 1) the study of the chemical polymorphism of aromatic endemic species; 2) the assessment of genetic fingerprint patterns of multiple populations to define genetic diversity and populations structure and correlate the genetic background with their chemical variability; 3) the evaluation of the biological properties of the essential oils from endemic species to determine their potential economic interest and 4) the consequent recognition of evolutionary "units" as source of genotypes, producing bioactive secondary metabolites, for eventual agriculture exploitation.

During the last three years (June 2007 to June 2010), within the frame of an FCT funded project, the essential oils from twenty aromatic endemic species were analysed by GC and GC-MS, in a total of 393 samples. The genetic profile of individuals from these species, using RAPDs and ISSRs as molecular markers, was compared with the corresponding chemical polymorphism. The biological activity of the isolated essential oils was screened for antimicrobial, antioxidant, anti-inflammatory and antiacetylcholinesterase properties as well as for cytotoxic activity using mammal and human cell lines.

Acknowledgements: FCT research contract PTDC/AGR-AAM/70136/2006.

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INDUSTRIAL BIOTECHNOLOGY

Oral Communications

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BEST (Bio-Esterification SynThesis) a versatile enzyme technology for multi-use applications

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The research programme “Biocatalysis/Enzyme Engineering” at BERG addresses the design and stabilization of enzymes and biocatalysts in order to improve their performance to specific industrial and diagnostic applications. One of the most interesting and promising field is on the enzymatic esterification reactions using cutinase and mutants thereoff with high activity and stability in non-conventional media (organic solvents and surfactants). Enzyme encapsulation or immobilization is in investigation to select the most appropriate enzyme technology platform, biocatalyst and reactor, for each specific and successful application.

Bio-Esterification SynThesis (BEST) is a versatile enzyme technology for multi-use application platform based on cutinase and engineered mutants and it is explored to address 4 major topics in ester biosynthesis of the topics highlighted below.

Aromase plataform purposes an alternative and innovative route for the synthesis of natural aroma ester compounds (flavors and fragrances – F&F) used largely in food, beverage, cosmetic and pharmaceutical industries,

Kirase platform in development seeks the chiral kinetic resolution of secondary alcohols which account for a very extensive area of applications mainly due to their importance in the synthesis of chiral building blocks and intermediates for the fine chemical and pharmaceutical industries,

Bio2diesel platform focus on the biodiesel production from vegetable oils or waste cooking oils or algae fatty acids and removable alcohols and can be an alternative manufacture route to the actual chemical synthesis using refined oils and methanol contributing to the biodiesel petroleum-based diesel with impact on the reduction of net greenhouse gas emissions,

Cerol-plastic platform is involved on the transesterification of vegetal oils or esterifition of glycerol a sub-product from the biodiesel industry with the goal to obtain glycerol derivatives as monomers for synthesis of bioplastics mainly water-borne polyurethanes and/or alkyd resins, which are largely used by Adhesives and Coatings Industries.

Chromatographic purification of plasmids with hydrophobic interaction membranes

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Plasmid DNA (pDNA) is becoming increasingly important as an active pharmaceutical ingredient for gene transference in gene therapy and DNA vaccination. Safety concerns with viral vectors were raised and pharmaceutical companies drove their attention to pDNA as demonstrated by the fast growing number of clinical trials with such bioproducts. These are still poorly immunogenic as compared to viral vectors and higher amounts of pDNA in its supercoiled topological form are required for a full treatment.

Cellular extracts containing the target pDNA have a diversity of other biomolecules that share structural and chemical properties with this therapeutic agent. Thus, highly selective and scalable purification processes are essential to provide large amounts of pDNA of pharmaceutical-grade quality at reasonable costs.

This presentation will outline the latest improvements on hydrophobic interaction chromatography process that was previously developed in our research group for the final purification step of pDNA [1, 2]. It focuses on the influence of the type of ligands in the dynamic binding capacity and selectivity of the HIC membrane adsorbers towards pDNA and host cell contaminants (*e.g.*, RNA and genomic DNA). A comparison of the performances of an alkylated membrane functionalized in our laboratory and a phenylic membrane provided by Sartorius Stedim Biotech GbmH will be presented.

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Ion Jelly® as a suitable optical transparent biomaterial for biosensing

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The development of new optical transparent biomaterials can open a window of opportunity for new applications in chemistry and biology, namely in biosensing. In the field of biosensors, a considerable research effort has been put into the development of efficient chemical reactions that can be monitored colorimetrically by combining the redox reaction with a dye-forming compound.[1] Based on this approach different strategies have been used to identify different metabolites, namely, hydrogen peroxide by the use of peroxidases. These assays can normally be carried out in two different ways; a) on solid phase or b) in liquid phase. The difference between these two strategies relies on different aspects, where the enzyme stability is one of the most important. Since many of enzymatic systems are not stable enough under chemical reaction conditions or present low stability over the time, the development of transparent matrix where the enzymes could retain their catalytic activity is of great importance. Taking advantage of the previous development on new matrix based on the combination of gelatine and ionic liquids that results on the formation of stable, transparent, flexible and conductor biomaterial (Ion Jelly®), the main goal of the present work is to develop a enzymatic colorimetric assay based on the use of optical transparent biomaterials that can be obtained from Ion Jelly® technology.[2] These optical transparent biomaterials have been use as a suitable matrix for immobilization of different enzymes, such as Glucose oxidase and Horseradish peroxidase.

Acknowledgments: We thank Fundação para a Ciência e Tecnologia for financial support (post-doctoral fellowships).

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Bringing miniaturization to bioconversion systems

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The introduction of miniaturized devices has been contributing for speeding up the development of bioprocesses, thus enhancing its competitiveness. Extreme miniaturization paved the way for a shift of paradigm in production strategies, allowing for scaling-out rather than scaling up. Regarding bioconversion systems, early steps towards miniaturization were anchored in simple microtiter plates (MTPs). MTPs provide a wide level of parallelization at microliter scale, hence allowing high throughput, and are prone of automation. MTPs rapidly evolved and became more sophisticated, either by incorporating monitoring and control devices, or through enhanced design for improved performance. Alongside, miniaturized reactors emulating larger vessels have been developed, as well as microstructured reactors. Together with such *hardware* developments, extensive knowledge have been gained for the engineering characterization of the systems operated in those devices (viz. fluid dynamics, scaling parameters) [1]. The whole gave rise to a plethora of platforms enabling: i) switching from the rather empirical process development approach - low throughput, lack of reproducibility and unsophisticated monitoring, if any - to high-throughput and reproducible experimentation, anchored in sophisticated monitoring; ii) shortening the path form process development of production scale. This work aims to provide an overview on key issues on these matters and on how the miniaturization approach was validated as a suitable approach for the study and characterization of multi-step bioconversion systems. In the case study selected, the microbial processing of steroids, operation with MTPs was fully characterized and scaling parameters established [2]. Furthermore, early steps towards operation in microfluidic environment were successfully undertaken.

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Capture of human monoclonal antibodies from cell culture supernatants by phenyl boronate chromatography

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Monoclonal antibodies (mAbs) hold great promise as new therapeutic agents against numerous diseases in an aging society, including the treatment of different type of cancers. Recent advances have lead to remarkable improvements in cell culture productivities, with antibody titers exceeding 10 g/l. With capacity bottlenecks moving towards downstream purification areas, the need for a broader strategic approach for the purification of mAbs is being increasingly recognized as the key to improve the overall process performance. Although several alternatives to the established downstream processing platform have been proposed, newer and more economic methods are still being pursued to facilitate the manufacturing of large amounts of mAbs that comply with the stringent impurity clearance requirements stipulated by regulatory agencies (FDA, EMEA).

Within this work, the feasibility of using phenyl boronate (PB) as an affinity ligand for the purification of mAbs has been investigated. The PB ligand is a useful tool for the specific capture and isolation of cis-diol-containing molecules, such as glycoproteins. Preliminary studies, using pure protein solutions, have shown that PB media can bind to human antibodies, not only at strong alkaline conditions but also at acidic pH values. On the other hand, insulin and human serum albumin (HSA), typical impurities found in cell culture media, did not bind at alkaline pH but at lower pH. Different binding and eluting buffers were evaluated for the capture of IgG from a CHO cell supernatant and the most promising results were obtained using 20 mM HEPES at pH 8.5 as binding buffer and 1.5 M Tris-HCl as eluting buffer. Using a step elution, all IgG was recovered in the elution pool with a maximum purification factor of 56 (in terms of total protein). A gradient elution allowed a further increase of the final purity, yet achieving a slightly lower yield. Purification factors for protein purity and HPLC purity were 76 and 39, respectively and IgG recovery was around 85% [1].

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Using adaptive mechanisms to improve bacterial performance

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The understanding of adaptation mechanisms in bacterial cells, including extremophiles, has allowed the use of new and extraordinary catalytic processes [1]. *Rhodococcus erythropolis* cells can tolerate and adapt to high concentrations of e.g. terpenes, hydrocarbons and aromatic compounds. The adaptation mechanisms include: i) alterations at the cell wall and membrane composition; ii) modifications of the physicochemical properties of the cell surface; iii) degradation or bioconversion of the toxic compound(s); iv) cell aggregation, and v) production of exopolymeric substances [2]. By adapting the cells in a biocatalytic system, it was possible to overcome the initial 50 mM carvone inhibitory concentration and to attain a final carvone concentration higher than 1 M. A similar strategy allowed the cells to metabolise concentrations of up to 4.9M toluene.

R. erythropolis cells could also be adapted to grow at 4 and 35ºC, at pH 4 and 11, at NaCl concentrations higher than 5% and at $CuSO_4$ concentrations as high as 1%. The adapted cells degraded both *n*-alkanes and alcohols under these conditions at good rates.

The production of storage lipids compounds under starvation conditions could be used as a source of triacylglycerols and fatty acids for biodiesel production. Biocracking of long chain alkanes was achieved in the presence of inhibitory compounds. Since these cells adapted the surface net charge according to the carbon source used, it was also possible to generate electricity between reactors containing the cells.

The results show the possibility of using adaptive mechanisms in biocatalysis and bioremediation processes and to produce bioenergy.

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Metabolomic approaches for the characterization of metabolic bottlenecks in recombinant protein production processes

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The optimization of bioprocesses using recombinant microorganisms is still restrained by the lack of information available on the metabolic responses induced by various stress conditions. The rapid exhaustion of essential metabolic precursors (e.g. amino acids) and cellular energy toward recombinant biosynthetic processes may result in the imbalance of the metabolism of the host cell, also called metabolic burden. In the past few years, the association of this metabolic burden with other cellular events, like the stringent response, has been demonstrated [1]. The unusual accumulation of ppGpp, a molecule produced by the ribosome-associated RelA synthetase induced by the deprivation of amino acids, is the hallmark of this stress response that results in the inhibition of cellular growth and lower productivity levels. The regulatory mechanisms of this ppGpp-induced response are known in some detail, but the impact of this response on the cellular metabolism has been less studied. Metabolomic analyses can provide substantial information at the biochemical level, in particular during recombinant bioprocesses. Therefore, metabolomic-based approaches [2], including profiling of intracellular and extracellular metabolite pools, were applied to investigate the influence of recombinant processes on the host cells' metabolism. In these studies two *E. coli* strains (*E. coli* W3110 and the isogenic ΔrelA mutant) were used to investigate the advantages of using "relaxed" phenotypes (i.e. ΔrelA mutant strain) as host cells in recombinant bioprocesses. Indeed, this cellular system presented major advantages in terms of biomass yield and productivity, which implied a remarkable improvement in recombinant bioprocesses.

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Yarrowia lipolytica: an industrial workhorse

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Yarrowia lipolytica is one of the most extensively studied ‘‘non-conventional’’ yeasts, being a strictly aerobic microorganism capable of producing important metabolites and having an intense secretory activity, which justifies efforts to use it in industry (as a biocatalyst), in molecular biology and in genetics studies. It is considered as nonpathogenic and several processes based on this organism were classified as GRAS by FDA, USA. This yeast is particularly adapted to hydrophobic substrates and in the last years it became a reference in research dealing with non-polar substrate metabolism. Many industrial applications of *Y. lipolytica* have been proposed, as shown by the many patents and papers mentioning it. One of the most important products secreted by this microorganism is lipase, which can be induced by many substrates, including olive mill wastewaters [1]. In fact, this yeast has been used for bioremediation applications due to its cell wall characteristics and surfactant production. In addition, *Y. lipolytica*, when grown under nutrient-limited conditions, is able to produce citric acid from a variety of carbon sources, including sugars, alkanes, plant oils, starch hydrolysates, ethanol, and raw glycerol (the main by-product of biodiesel production units). The production of aroma compounds from fatty acids though β-oxidation machinery of *Y. lipolytica* has been also extensively studied [2]. Moreover, this yeast as been described as oleaginous, with great potential to be used as single cell oils (SCO). Being strictly aerobic yeast, its growth and metabolite secretion are affected by the amount of oxygen available in the culture medium. Thus, studies of oxygen transfer mechanisms into such complex media systems composed of oil-in-water emulsions have gained great interest.

This work aims to throw light on how a single organism can be versatile with respect to its metabolic abilities, being exploited for a variety of purposes.

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Application of a statistic tool for on-line characterization of bubble population complexity in a multiphase reactor

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Image analysis technique has been proved to be very effective in the quantification of particles size and morphology distributions in different work areas. In the present work this technique was combined with the Discriminant factorial analysis (DFA) in order to allow the identification of single bubbles (isolated bubbles without influence of surrounded bubbles) and to study the bubble population complexity in multiphase reactors. By this way, it is possible to determine correctly the average bubble size and, consequently, the specific interfacial area a on the different experimental conditions. With the previous methodology it has been also possible to distinguish on-line and automatically among three different classes of bubbles, allowing the computation of the bubble population complexity in the system through the new parameter, the complexity degree of bubbles. Agreement between automated and manual classification, measured in terms of a performance index, is 90% on average. Further, it describes the application of such methodology to the study of the influence of bubble characteristics (size, shape, bubble population complexity, etc) on the individual parameters of volumetric liquid side mass transfer coefficient, kLa. The experiments were done at different temperatures (25-35ºC) and superficial gas velocities (up to 14 mm/s) in a bubble column.

Isolation of microorganisms from oil samples for application in microbial enhanced oil recovery

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Microbial Enhanced Oil Recovery (MEOR) is an important tertiary oil recovery process where microorganisms and their metabolites are used to retrieve unrecoverable oil from a reservoir after application of primary and secondary recovery techniques [1]. Stimulation of bacterial growth and biosurfactant production by indigenous microorganisms can reduce the capillary forces that retain the oil into the reservoir. MEOR offers major advantages over conventional EOR, namely low amounts of energy consumption and independence of the price of crude oil. In this work we have been addressing the isolation and identification of microorganisms capable of producing biosurfactants under conditions existent in oil reservoirs. Biosurfactant production by microorganisms isolated from crude oil samples was evaluated by measuring surface tension and emulsification activity. Among the isolated microorganisms, seven *Bacillus* strains were able to grow and produce extracellular biosurfactants at 40ºC under anaerobic conditions in medium supplemented with hydrocarbons. Three isolates (PX309, PX311 and PX573) were selected as the higher biosurfactant producers; biosurfactants produced by those isolates reduce the surface tension of water from 72 to 30 mN/m, exhibit emulsifying activity and are not affected by exposure to high temperatures (121ºC), which make them good candidates for use at the extreme conditions usually existent in oil reservoirs. The results obtained show that those isolates exhibit potential for the development of enhanced oil recovery processes.

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Identification of genes and process conditions required to improve alcoholic fermentation yield under industrially relevant fermentation media

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The successful performance of alcoholic fermentations relies on the ability of *Saccharomyces cerevisiae* strains to cope with multiple stress factors occurring during the fermentation process and on the selection of suitable process engineering strategies [1].

An optimized very high gravity (VHG) glucose medium [2] containing low cost nutrients was developed for stress-tolerant and high fermentation efficiency yeasts selection from “cachaça” and bio-ethanol production plants in Brazil [3]. CA1185 and PE-2 strains, which produce remarkably high ethanol titres (>19%, v/v), are physiologically more prepared to cope with VHG stresses relatively to laboratory strains. Recent results show that this robustness is, most likely, related to specific features of yeast cell wall and plasma membrane. Considering the outstanding properties of PE-2 strain, this yeast strain was genetically modified to introduce flocculation and used in repeated batch fermentations with high cell density resulting in significant improvements on process economics.

A genome-wide screening for determinants of yeast resistance to high sugar [4] and ethanol [5] stresses, relevant in VHG technology, and to inhibitory fermentation compounds from lignocellulosic pre-treatments, including acetic acid [6], was also carried out. Eighteen genes were identified as determinants of yeast resistance to more than three of the above mentioned fermentation relevant stresses. Among these genes, 8 were found to significantly affect ethanol kinetics and/or production in VHG and hemicellulosic liquor fermentations mimicking industrially relevant conditions. Guided by all the gathered information extraordinarily robust strains are being designed to be used in efficient and economic industrial alcoholic fermentation processes.

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The use of bacteriophage endolysins as antibacterial compounds

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The widespread use of antibiotics to eliminate pathogenic microorganisms is currently under pressure, due to the alarming emergence of antibiotic resistance. This has forced scientists to widen their scope in the search for alternatives, and has drawn the attention to bacteriophage (phage) as new bactericidal entities. Phages are viruses that highly specifically recognize and infect bacteria. After invasion and multiplication of the host, phage can turn lytic by hydrolyzing the host´s cell wall, using (endo)lysins. Lysins are hydrolytic enzymes that generally consist of two domains: a catalytic domain (e.g. N-terminal) and a C-terminal cell binding domain (CBD) that binds to a specific sugar molecule on the cell wall. The idea of using lysins as stand-alone active compounds has only been considered since recently [1]. Several reports show that the external addition of lysins to Gram-positive pathogens (*Bacillus anthracis*, Streptococci) results in an effective *in vivo* elimination of the organism, similar to the extent whole phages would kill them [2]. This demonstrates that purified lysins from phages serve efficiently as antimicrobials.

The access to a range of lytic bacteriophages and new phage isolates active against several pathogens has allowed us to screen their genomes and select for endolysins. Some were heterologously produced and purified, showing a similar activity range as their corresponding phages. Moreover, we are currently isolating endolysins from phages active against Gram-negative bacteria and developing strategies to apply them directly, without the need of pretreatment of the host´s outer membrane.

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Intact-cell MALDI-TOF (ICM) mass spectrometry for rapid identification and subtyping of *Burkholderia cepacia* complex bacteria

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Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS) analyses the chemical cellular composition of microorganisms providing rapid, discriminatory fingerprints for identification and subtyping of important nosocomial pathogens. The remarkable reproducibility of this technique is based on the measurement of constantly expressed and highly abundant proteins. The usually observable molecular mass range is between 2,000 and 20,000 Da, where important ribosomal proteins appear, which is an advantage because these can be easily used as biomarkers. The *Burkholderia cepacia* complex (Bcc) comprises at least 17 closely related bacterial species that have very high metabolic versatility, are ubiquitous in the environment and can cause opportunistic infections, in particular in patients with cystic fibrosis (CF). Chronic respiratory infections caused by these bacteria are, in general, characterised by low responsiveness to antibiotic therapy and rapid reduction of lung function. Epidemiological surveys of Bcc bacteria involved in respiratory infections among the Portuguese CF population under surveillance at this Pediatric and Adult CF Treatment Center of Santa Maria Hospital (HSM), in Lisbon, have been carried out by the IST laboratory, covering isolates obtained since 1995. They belong to *B. cenocepacia* (*recA* lineages IIIA and IIIB), *B. cepacia*, *B. multivorans* and *B. stabilis* species, with an exceptionally high representation of *B. cepacia*. These isolates were classified at the species level by established molecular methods, and differentiated at the strain level, based on their ribopattern/multilocus sequence typing (MLST) profiles. However, these techniques are time-consuming and expensive. In order to overcome these limitations MALDI-TOF ICMS was successfully explored as a rapid, precise, and cost-effective tool for identification and subtyping of intact Bcc bacteria. The method was tested using isolates obtained in the HSM CF Center, including clonal variants retrieved from the same CF patients during several years of chronic colonisation, previously characterized by conventional molecular biology techniques.

Evaluation of the potential of hierarchical starch-based fibrous scaffold for bone tissue engineering applications

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Knowledge on cell transplantation, materials science and bioengineering has led to the development of biomedical devices capable of restoration and maintenance of normal function upon implantation in diseased and injured tissues. However, there are still many challenges in developing an efficient strategy for bone tissue engineering. Rapid prototyping (RP) techniques allow the design and construction of complex polymeric structures with different shapes and sizes. Despite the regular and completely interconnected pore network that characterizes RP scaffolds, cell seeding efficiency still remains a critical factor for optimal tissue engineering applications. Hierarchical fibrous scaffolds, obtained by the combination of RP micro- and electrospun nano-motifs, were developed to overcome this drawback [1]. Bone marrow mesenchymal stem cells (hBMSCs) are a very promising source of cells for human tissue engineering strategies. Recent evidences shown that human Wharton's jelly stromal cells (hWJSCs) are a primitive stromal population sharing similar characteristics with hBMSCs [2]. In this study, the potential of hierarchical starch-based fibrous scaffolds for bone tissue engineering is qualitatively and quantitatively evaluated *in vitro*.

SEM micrographs show that hWJSCs preferentially adhered to the nanofibrous meshes of RP+NFM scaffolds. Biological data demonstrated that hWJSCs presented significantly higher cell proliferation and maturation when seeded onto hierarchical starch-based fibrous scaffolds. Furthermore, up-regulation of bone specific genes and calcium phosphate deposition confirmed the successful osteogenic differentiation of hWJSCs on these scaffolds. These results corroborate our hypothesis that the integration of nanoscale fibers into 3D rapid prototype scaffolds substantially improves their biological performance in bone tissue engineering strategies.

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Functionalized Silk Biomaterials for Bone Formation and Infection Control

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Our main goal was to develop new functionalized silk-based biomaterials with mineralization and antimicrobial domains. By exploiting these functional domains we are able to induce osteogenesis and to control bacterial infection. New chimeric silk proteins were generated via recombinant DNA techniques, combining spider silk from the consensus repeat of major ampullate spidroin protein I (6mer) with different functional proteins, namely: bone sialoprotein (BSP) and hepcidin, human neutrophils defensins 2 (HNP-2) and 4 (HNP-4). BSP is involved in the deposition of calcium phosphates and binding to hydroxyapatite and collagen I. Hepcidin, HNP-2 and 4 HNP-4 have proved antimicrobial activity. These new functional biomaterials combine the useful features of the silk protein component, such as self-assembly, mechanics, aqueous processing, with the new features from the added peptides and proteins, such as improved bone integration and infection control. For antimicrobial activity the inhibition of *Escherichia coli* and *Staphylococcus aureus* was assessed based on zones of clearance on agar plates using filter paper disks soaked in the different chimeric proteins at different concentrations. 6mer was used as control. The zones of clearing confirmed the functional features of the antimicrobial peptide-silk chimeras. For the BSP, mineralization assays were conducted in vitro to assess function. Based on scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS) calcium phosphate formation on the chimeras was confirmed, while similar outcomes were not found for the control materials (6mer). For the new biomaterials cell viability and proliferation was also assessed.

Cork based composites as core in flooring applications: Characterization and optimization process using experimental design

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The cork industry produces high amounts of cork powders resulting from the final stages of cork processing without a significant economical value. Cork is a natural, renewable and sustainable raw material [1] with an unexploited potential to be used on the development of partially or completely natural based composites [2]. Cork powder (50wt.%) was mixed with polyethylene (PE) and polypropylene (PP) by pultrusion aiming to prepare cork based composites. In a further step, samples were produced by compression moulding using the compounded composites. Impact resistance, hardness, dimensional stability and acoustic properties of the prepared composites were determined and compared with commercially available products namely medium density (MDF) and high density (HDF) fibreboards. It was found that the cork based composite have higher dimensional stability, lower water uptake, higher performance regarding acoustic isolation and similar behaviour in terms of hardness when compared with MDF and HDF. Additionally cork improved the energy absorption and the hardness of the polyolefin matrixes. Factorial design was used to optimize the compression moulding process in order to obtain the best mechanical performance parameters. The developed cork polymer composite (CPC) materials showed important characteristics to be applied in the design of flooring and construction systems.

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Polysaccharide-based carriers for mucosal protein delivery

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Protein-based drugs demonstrate great therapeutic potential, but their delivery is still a major challenge due to specific physicochemical properties, poor stability and permeability. Although parenteral delivery remains the primary choice for protein delivery, mucosal administration has turned an attractive alternative. Successful administration is, thus, highly dependent on the development of suitable carriers. In this context, polymeric vehicles like micro and nanoparticles have been gaining popularity, because they provide protection to the encapsulated molecule, can be tailored to specific needs and are frequently associated to a more controlled release when compared to conventional forms [1]. In fact, these systems provide increased therapeutic effect by the reduction of dosage frequency, which is accompanied by a decrease of side effects. Natural-origin polymers are frequently selected to compose these drug delivery systems, as they easily comply with the mandatory requisites of biocompatibility, biodegradability and absence of toxicity. Polysaccharides like chitosan, carrageenan, starch and locust bean gum have been increasingly used in drug delivery research, as a result of promising properties like bioadhesion [2].

The objective of this work was to develop polysaccharide-based micro and nanoparticles to be used as protein carriers for mucosal administration.

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Chimerical nanoparticles as therapeutical vehicles

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New virus-like nanoparticles (VNPs) have been considered in vaccination, gene therapy and drug delivery approaches. VNPs can be produced by heterologous expression in a high range of host cells and can be engineered to present different signal molecules at the particle internal and external surfaces, which can be further explored to enhance the encapsulation of therapeutic agents, to target specific cells or tissues, or to stimulate humoral or cytotoxic responses. Our lab has been working in the production and purification of complex self-assembled nanoparticles from cell cultures, and also in its manipulation in order to address different therapeutic targets.

A chimeric Simian – Human Immunodeficiency virus-like nanoparticle was constructed by fusion of SIV matrix protein (p17) and HIV-1 p6 protein. This fusion protein assembles as spherical nanoparticles of about 80 nm in diameter which are released to the culture medium when expressed in HEK 293T cells [1].

Purification of these particles is achieved by a simple two step purification system: an ultrafiltration/diafiltration step followed by an ion-exchange chromatography.

Our therapeutical targets include: i) anti-HIV1 therapy, by targeting infected cells and promote the delivery of specific antibodies against a key protein in this viral infection; ii) delivery of transcription factors for cell reprogramming, overcoming existing challenges when using lentiviral vectors; iii) target cancer cells *in vivo* with specific domains and promote tumour regression.

Acknowledgement to the funding: Projects - IBB/CBME, LA, FEDER/POCI 2010, PTDC/BIO/69682/2006, and PTDC/SAU/ENB/111702/2009; Grants: SFRH/BD/36674/2007 and SFRH/BPD/30290/2006.

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A new synthesis approach to control acidity in SAPO materials: Use of methylamine as co-template

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Since the first report of the existence of silicoaluminophosphate molecular sieves (SAPOs) by Lok et al. 0, more than 57 different frameworks have been recognized by the IZA Structure Commission and extensively investigated in relation to their interesting catalytic properties. Because of its weak to moderate Brönsted acidity and shape selectivity, the medium pore SAPO-11 (AEL) has been proved to be excellent candidate for isomerization of paraffins and it is currently used in the lube oil dewaxing Chevron Process. The acidity of such material is currently achieved by inserting Si atoms into the $AlPO_4$ framework, generating negative charges that are balanced by protons attached to Si-O-Al bridges (mechanism SM2). Depending on the preparation conditions and the initial Si contents, the double substitution of neighboring Al and P by two Si can also occur (mechanism SM3), thereby leading to extended Si islands and various acid site environments with variable number and strength 0. The present work consisted of modifying the synthesis procedure of SAPO-11 materials, through the use of dual templates, dipropylamine (DPA) as main template and a short chain amine, methylamine (MA), selected as co-template. Methylamine, although rarely used as sole template in microporous $AlPO_4$ syntheses, often significantly influences the synthesis mechanisms of silica-based porous materials in various ways. The results obtained from chemical analyses, TG measurements, ^{29}Si NMR and Infrared spectroscopy showed that MA was capable to easily modify Si distribution within the AlPO framework, giving rise to heterogeneous catalysts with enhanced Brönsted acidity properties.

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Bio-oils and FCC feedstocks co-processing: impact of guaiacol on n-heptane transformation over acid zeolites

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To reduce petroleum dependence and to avoid climatic changes, new energy sources able to replace fossil fuels should be found. Lignocellulosic biomass is, currently, the only renewable source of carbon that can be easily converted into liquid fuels (bio-oils) to be used as transportation fuels [1]. However, the amount of oxygenated compounds in the bio-oils is high [1]. A possibility to produce bio-fuels could be to co-feed the bio-oils with the conventional Fluid Catalytic Cracking (FCC) feedstocks. A hydrodeoxygenation treatment (HDO) should be envisaged before the co-feeding [2]. Nevertheless, phenolic molecules are particularly refractory to HDO [1], and they still remain in the bio-oils that would be introduced in the FCC units.

To evaluate the possibility to partially replace the classical FCC feedstoks by bio-oils, the n-heptane transformation was performed in presence of guaiacol over HY and HZSM-5 zeolites (active parts of FCC catalyst), at 350 and 450ºC. Whatever the temperature, guaiacol leads to a further deactivation that is observed since the beginning of the reaction, resulting from an increase of the carbonaceous materials retained on the zeolite pores due to guaiacol adsorption on the acid sites. Its deactivating effect is more pronounced for the HZSM-5. Higher temperatures disfavour guaiacol adsorption and retention on both HY and HZSM-5 zeolites, principally for the HZSM-5. Guaiacol conversion into phenol with loss of the metoxy- group as CH_4 and water is a reaction that takes place over these zeolites, but, under the used operating conditions, it occurs only to a lower extent.

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HEALTH BIOTECHNOLOGY

Oral Communications

Degradation Kinetics and Host Response of Chitosan based Scaffolds

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Previous studies showed that chitosan-poly(butylene succinate) fiber mesh scaffolds evidenced significant biological performance [1,2]. Nevertheless, in a tissue engineering approach it is crucial to determine the kinetics of biodegradation of the biomaterials *in vitro*, as well as *in vivo.* Furthermore, the host response to these biomaterials must be evaluated to understand the extent of the inflammatory response to not compromise the referred application.

This study reports the degradation process *in vitro* using relevant enzymes (lipase and lysozyme) involved in the degradation of the two polymers. We used concentrations similar to those found in the human body, in dynamic and static conditions changing or not the solutions. Water uptake and weight loss measurements were performed. In addition, subcutaneous implantation of the scaffolds was performed to assess by histology the type of inflammatory cells present in the surrounding tissue, as well as inside the scaffold structure.

In the presence of lipase, or with lysozyme, water uptake increased. This phenomenon is probably due to the degradation of scaffolds by the enzymes. Weight loss results evidenced that lysozyme and lipase have a notable effect on the degradation of the scaffolds *in vitro*, loosing its structural integrity after 6 weeks.

In vivo implantation showed a normal and mild inflammatory response with the typical presence of neutrophils in a first stage and macrophages, lymphocytes and giant cells in a later stage. Moreover, neo-vascularization was observed, increasing with time, by the presence of new blood vessels in the surrounding tissue and inside the implant.

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Novel hydrogels based on carrageenan with encaspulated adipose derived stem cells for cartilage tissue engineering

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The use of injectable hydrogels for cell delivery in cartilage regeneration therapies have attracted more and more attention because of their promising advantages over pre-formed scaffolds [1]. Temperature-dependent natural biopolymer k-carrageenan hydrogel has been investigated as a new injectable system [2]. The present work focuses on evaluating the swelling and mechanical properties of k-carrageenan hydrogel as well as their ability to support the viability, proliferation and chondrogenic differentiation of encapsulated human adipose derived stem cells (hASCs) For this purpose, we examined the water uptake in different swelling mediums and the mechanical properties of the hydrogels with cells encapsulated cultured for up to 21 days. The phenotype profile of encapsulated hASCs, cultured either in basal or chondrogenic medium, was analysed using real time RT-PCR analysis, for specific cartilage markers (SOX-9, Col II, Col I, Col X, Aggrecan) and immunohistochemical analysis (Col II and Col I immunolocalization). The results obtained indicate the feasibility of using k-carrageenan gel as a cell carrier and as potential injectable systems, due to its swelling and mechanical properties and due to its ability to maintain viability and induce chondrogenic differentiation of encapsulated human adipose derived stem cells.

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Improved cell adhesion on distinct biomedical devices via surface incorporation of negatively charged functional groups

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Biocompatibility of natural carbohydrates (eg. chitosan) is often associated with the structural similarities with glycosaminoglycans (GAGs). Although all of the GAGs are built from repeating disaccharide units and some of them contain N-glucosamine (the main hexosamine in the chitosan backbone), all of them do also contain negatively charged functional groups. These charged units are believed to have a crucial role for the formation of proteoglycans and hence for key biochemical processes/signalling related to cell functionality and survival. Thus, we reported that the presence of anionic functionalities such as phosphonic [1, 2] and sulfonic groups [2] can induce remarkably distinguishable cell response through the adsorbed proteins from the culture medium and significantly improves the adhesion, proliferation and viability of osteoblast cells. The negatively charged groups were introduced by plasma induced grafting of phosphonic and sulfonic acids, respectively. The main advantage of the proposed method is that the surface properties can be enhanced selectively, while the bulk attributes (eg. biodegradability) of the materials remain unchanged. Besides its simplicity and versatility, this method can be easily applied to different materials and structures with complex shapes such as scaffolds for tissue engineering applications.

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Injectable Gellan gum-based hydrogels for intervertebral disc regeneration

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Intervertebral disc (IVD) degeneration is a challenging pathology that, due to the inefficiency of the current treatments, urgently demands for the development of new tissue engineering approaches [1]. The best viable implant material for nucleus pulposus (NP) regeneration has yet to be identified, but it is believed that biodegradable hydrogel-based materials are promising candidates [2]. We are proposing in this work the use of ionic- and photo-crosslinked methacrylated Gellan gum (GG-MA) hydrogels as potential acellular and cellular injectable scaffolds for IVD regeneration. The developed hydrogels were physico-chemically characterized by Fourier-transform infrared spectroscopy, ^{1}H nuclear magnetic resonance and differential scanning calorimetry. Hydrogels swelling capacity and degradation rate were also analyzed in a phosphate buffered saline solution at physiological pH, for the period of 30 days. Additionally, the morphology and mechanical properties of the hydrogels were assessed under a scanning electron microscope and dynamic compression analysis, respectively. Citotoxicity of the Gellan gum-based hydrogels leachables was evaluated by carrying out a cellular viability assay (MTS test) on rat lung fibroblasts (L929 cell line) cells, which were previously in contact with the different extracts for the period of 24 hrs until 7 days. Results demonstrated that Gellan gum was successfully methacrylated and the produced GG-MA hydrogels possess improved mechanical properties and lower water uptake ability and degradation rate as compared to Gellan gum. This work also revealed that GG-MA hydrogels are non-cytotoxic *in vitro*, thus being promising biomaterials to be used in IVD tissue engineering strategies.

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New multilayer approach for producing moldable 3D nanostructured constructs from random spheres to be used in regenerative medicine.

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In tissue engineering scaffolds having controlled 3D geometry are needed for temporary support of cells. Layer by layer (LbL) templating technique has been widely used to prepare nanostructured coating with tailored properties but mostly limited to flat surfaces.[1] In this work we intend to expand it to prepare scaffolds.[2] The concept is based on the use of packed particles acting as templates, which are leached out after being coated with a multilayer biocompatible film. Sodium alginate, chitosan, paraffin wax spheres, poly ethylenimine (PEA), sodium chloride, dichloromethane were used. Solutions of 1mg/ml Sodium alginate and Chitosan concentration in 0.15M NaCl were adsorbed sequentially, followed by washing over the PEA coated spheres (0.5 wt % solution in water) in a free standing packing over a perforated cylinder assembly. After 10 bilayer coatings, DCM was used for removing wax beads followed by freeze drying. Osteoblast-like cells were cultured for 7 days. Florescence, confocal and scanning electron microscopy (SEM), micro CT were used for characterization. Perfusion technique was used in place of dipping or spraying to the build up of the multilayers. The packing of paraffin wax spheres, critical to the formation of the 3D structure, remained stable throughout the experiment. Fine 3D multilayer network exhibiting interconnectivity was obtained after leaching and freeze drying leading to desired scaffold formation. They were able to support cell attachment and proliferation even in the inner regions of the structure. In conclusion a new methodology was presented to fabricate scaffold entirely constituted of nanostructured multilayers.

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CMCht/PAMAM Dendrimer Nanoparticles as Cell Specific Drug Delivery Systems for Spinal Cord Injury Applications

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Spinal cord lesions induce a cascade of biochemical responses that create an unsuitable environment for the nervous tissue regeneration. Current therapies are based on pharmacological agents that present low efficacy. Being so, novel strategies such as cell targeted drug delivery systems have to be considered. A recently described system based on a dendrimeric poly(amidoamine) (PAMAM) core grafted with carboxymethylchitosan (CMCht) [1] has shown to be amenable for the delivery of drugs to nervous cells [2]. Thus, the aims of this work were to: i) load the CMCht/PAMAM nanoparticles (NPs) with methylprednisolone (MP) and test its influence in glial cell cultures; ii) modify the surface of the NPs rendering them a targeted profile; and iii) study the *in vitro* cell internalization specificity of the NPs. To achieve so, CMCht/PAMAM dendrimer NPs were produced [1] and MP was incorporated by a precipitation route. A microglia specific CD11b antibody was linked to the NPs by a crosslinking reaction. Cortical glial cells were isolated from P4 male Wistar rats to perform the *in vitro* studies and the NPs were added to the cultures every 48 hours for a week. Cell viability was assessed using the MTS assay and immunocytochemistry was performed for different glial cell types to assess the internalization rates. FTIR spectroscopy confirmed the MP incorporation and ^{1}H NMR indicated the antibody linkage. MP sustained release profiles were detected by HPLC analysis (0-14 days). The *in vitro* experiments and fluorescence microscopy images revealed that the CD11b-NPs internalization was significantly reduced in astrocytes and oligodendrocytes while maintaining high internalization rates in microglial cells.

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Development of enhanced low-serum culture strategies: Its effect on the "stemness" profile of Amniotic Fluid Stem Cells

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Mesenchymal stem cells (MSCs) are an appealing cell population for cell-based therapies and tissue engineering. However, its frequency is low and requires expansion before its use in clinical applications. Many studies already demonstrate MSCs potential both for cartilage and bone repair, in promoting engraftment and in the treatment/prevention of graft-versus-host disease. In most of those studies, the cells are expanded in serum-containing medium, although, to meet the regulations required by clinical applications, the elimination of serum is highly desirable [1, 2]. Thus, the aim of this work is to evaluate the effect of low-serum culture conditions on the MSCs genes profile of human amniotic fluid stem cells (hAFSCs). hAFSCs were cultured in media containing 2%, 5% or 10% foetal bovine serum (FBS). The effect of FBS concentrations in the culture medium and the passage number on the expression of CD105, CD73 and CD90 were evaluated by quantitative RT-PCR. No differences were observed in the gene profile with increasing of cell passage, suggesting that hAF FSCs maintain their "stemness" with culturing time. Expanded cells in low-serum culture conditions showed higher expression of stem cell markers when compared with their normal-serum counterpart cultures. Moreover, the osteogenic potential is not impaired after expansion in low-serum conditions. We have successfully expanded hAFSCs in low-serum culture (2% and 5%), with increase expression of markers responsible for keeping multlineage differentiative potential. With this work we aimed to contribute to the characterization of hAFSCs populations, providing evidence that expansion of those cells to obtain relevant numbers is possible, envisioning their prospective use in new regenerative medicine strategies.

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Evaluation of amniotic fluid stem cells and biodegradable starch-polycaprolactone scaffolds for the regeneration of bone non-union defects

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Bone tissue engineering (bTE) requires cells with high proliferative and osteogenic potential, and a scaffold to support cell development towards bone formation. Amniotic fluid stem cells (AFSCs) have been differentiated into multi-lineage cells, including the osteogenic lineage[1]. Starch-polycaprolactone (SPCL) blends are degradable and biocompatible and showed interesting results for bTE applications[2]. We hypothesize the stage of osteogenic differentiation of AFSCs, when seeded onto SPCL scaffolds, may affect the bone regenerative process in femoral non union defects of rats.

SPCL scaffolds, produced by a fiber melting technique[2], were cut into discs and ethylene oxide sterilized. AFSCs were seeded onto SPCL scaffolds and cultured for different periods in osteogenic medium to have i) undifferentiated cells, ii) pre-osteogenic cells committed to the osteogenic phenotype and iii) "osteoblastic-like" cells. After reaching each stage of differentiation, samples were characterized for cellular viability, osteogenic phenotypic expression and matrix formation. These constructs were implanted in critical sized femoral defects in nude rats and bone formation was evaluated after 4 and 16 weeks post-implantation by microCT and histological markers.

AFSCs showed osteogenic commitment after 2 weeks, and successfully differentiated into bone-like cells after 21 days in osteogenic culture, as confirmed by alizarin red, alkaline phosphatase staining, and collagen I immunofluorescence. Mineralized extracellular matrix (ECM) was detected in constructs after 3 weeks in osteogenic culture.

In vivo characterization studies are still ongoing. Nevertheless, preliminary mCT data indicates that bone formation is more evident in SPCL and AFSCs-SPCL constructs, while the bridging effect between the bone segments is improved in SPCL seeded with osteogenic committed AFSCs.

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Non-Viral Gene Delivery to human Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) hold a great promise for application in several therapies due to their unique biological characteristics. In order to harness their full potential in cell-or gene-based therapies it might be advantageous to enhance some of their features through gene delivery strategies. Accordingly, we are interested in developing an efficient and safe methodology to genetically engineer human bone marrow MSC (BM MSC), enhancing their therapeutic efficacy in Regenerative Medicine. In our work the delivery of plasmid DNA was optimized using lipofection and a recently available microporation technique. Emphasis was given not only to the percentage of transfected cells but also to the cell recovery and we verified that higher number of transfected cells gave rise to higher levels of cell death. Even though with 40% of transfected cells it was possible to attain 90% of cell recovery which surpasses the yields of transfection presented so far with MSC. After lipofection or microporation, with or without plasmid, cells showed a lower clonogenic potential although no effect was observed in the immunophenotypic characteristics or differentiative potential of transfected cells. Importantly, the number of plasmid copies in different cell passages was quantified for the first time and 20,000 plasmid copies/cell were obtained independently of cell passage, after lipofection [1]. Moreover, The BM-MSC proliferation kinetics were mainly affected by the presence of plasmid and not due to the gene delivery process itself [2].
With our work we may assume that both lipofection and microporation are reliable and efficient methods to genetically modify hard-to-transfect cells giving rise to the highest levels of cell survival reported so far concurrently with superior gene delivery efficiencies.

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Different stages of pluripotency determine distinct patterns of proliferation, metabolism, and lineage commitment of embryonic stem cells under hypoxia

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Mouse embryonic stem (ES) cells are routinely cultured in vitro under atmospheric oxygen levels (20% O_2). However, mammalian cells are usually exposed to lower oxygen tensions in their niche. Therefore, it is critical to understand how low oxygen tensions influence the growth and metabolism of stem cells cultured in vitro in order to develop novel approaches for the large-scale bioprocessing of pluripotent stem cells and their progeny [1].

Herein, we have studied the effect of low oxygen levels (2% O_2), or hypoxia, in the expansion and neural commitment of mouse embryonic stem (ES) cells. In the presence of leukemia inhibitory factor (LIF), cell proliferation was reduced under hypoxia and a simultaneous reduction in cell viability was also observed. Slight morphological changes were registered suggesting some early differentiation for hypoxic conditions. However, when cells were maintained in a ground state of pluripotency [2], by inhibition of autocrine FGF4/ERK signaling, hypoxia did not affect cell proliferation, and did not induce early differentiation. The proliferation of mouse ES cells under these conditions was not affected, independently of the oxygen level used. In addition, over 95% of cells were positive for Oct-4 and Nanog, either at low or at atmospheric oxygen tensions, as quantified by flow cytometry. During neural commitment, low oxygen tension exerted a positive effect on early differentiation of ground state ES cells, resulting in a faster commitment towards neural progenitors. Using a reporter ES cell line, it was possible to examine the process by which ES cells acquire neural identity through expression of Sox1. This revealed that the maximum expression of Sox1 was reached faster at 2% O_2, and by day 4 cells maintained under low oxygen levels were already over 80% Sox1-positive.

Overall our results demonstrate the need to specifically regulate the oxygen content, along with other culture conditions, when developing new strategies for ES cell expansion and/or controlled differentiation.

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Structural instability in plasmid biopharmaceuticals for DNA vaccination

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DNA repeats are considered to be catalysts of molecular evolution by promoting genetic instability and mutational events. One of the concerns associated with the presence of repeated elements, consists in the occurrence of recombination events within plasmids used for biopharmaceutical applications such as gene therapy and DNA vaccination [1]. In the recent years several authors have found evidence for structural instability phenomena occurring spontaneously in plasmids used for DNA vaccination, including repeat-mediated deletion-formation and transposition of mobile elements (reviewed in [1]).

In this work we present an overview of the main achievements and contributions of our group in the field of plasmid structural instability. Particularly, we present data on the identification of several genetic aberrations in backbones used for DNA vaccine development and how the latter are affected by environmental stresses typically found during amplification. A meta-analysis-driven mathematical tool for recombination frequency prediction in plasmids was also developed, which takes into account the influence of strain genotype in terms of its *rec*A function. Finally, we present the results of an *in silico* search for interspersed repeated regions with high recombination potential, performed in a large number of commercial vectors. This analysis led us to conclude that these hotspots are widespread, even in plasmids currently used for DNA vaccine development, and further allowed the detection of a novel recombination event in the widely used human cytomegalovirus immediate early enhancer/promoter [2]. Altogether, these findings are crucial in understanding the real extension of unstable hotspots in plasmid vectors.

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Molecular mechanisms controlling pluripotency and differentiation of embryonic stem cells, and improvement of cell reprogramming

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New cellular therapies aiming to correct dysfunctions of tissues and organs were tested in preclinical studies with promising results. Embryonic stem cells (ESC), with the ability to proliferate rapidly and efficiently in culture, and the capacity to give rise to every cell type that constitutes an organism, represent an unlimited supply of cells for therapy future cell-based therapies for treatment of degenerative diseases, genetic disorders, or injuries due to inflammation, infection and trauma. Induced pluripotent stem cells (iPS) with properties indistinguishable from those of ESC were obtained by reprogramming adult cells, and have attracted great interest in the scientific and medical communities as an alternative cellular source to human ES cells, for the development of personalized therapies. However, risks of developing cancer associated with the pluripotent nature of ESC and iPS, the reprogramming process, as well as the immune rejection of cells genetically unrelated to the patient, and the inefficiency and unreliability of differentiation processes keep holding back their clinical application. Thus, understanding the molecular mechanisms involved in the self-renewal, reprogramming and differentiation processes of ESC and iPS cells is essential to overcome these issues. Our group is focusing on understanding the role played by the transcriptional co-activator Cited2 in these processes.

Timing cell specification in embryo development

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Embryo development proceeds under strict spatial and temporal control. This is particularly crucial during gastrulation, when collinear activation of *Hox* genes specifies cell identity/positional information by timing progressive epiblast cell ingression in the primitive streak [1]. However, the mechanisms underlying temporal collinearity of *Hox* gene activation are far from being understood.

An Embryonic Molecular Clock (EC) was first evidenced by cyclic *hairy1* gene expression in presomitic mesoderm [2] and is now known to operate in many different tissues/cells. Brachyury (T) protein, essential for *Hox* gene expression and mesodermal cell migration through the primitive streak, was identified as a Hairy1-interacting protein by yeast two-hybrid. This was independently confirmed and visualized *in vivo* using Bimolecular Fluorescent Complementation (BiFC) and strongly suggests that the EC could be coupling *temporal* and *positional* information.

According to our model, cyclic Hairy1-T dimer assembly/disassembly could be timing *Hox* gene activation and ultimately, cellular specification in development. To test this, *hairy1* or *T* were over-expressed in early embryos by electroporation. We found that over-expression of either gene delays or even prevents somite formation, to the same extent. A dose-dependent phenotype was obtained with the C-terminal, T-interacting domain of Hairy1, suggesting a titration effect. Moreover, we have characterized the *HoxB* cluster expression profile during chick development by RT-qPCR. Upon *hairy1* or *T* over-expression, *hoxB* expression was significantly delayed, further supporting our model.

Altogether, our data suggest that the EC is timing cell specification by regulating temporal collinearity of *Hox* gene expression initiation, mediated by cyclic Hairy1-T protein complex formation.

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A novel pathway to oxidative protein folding in the endoplasmic reticulum

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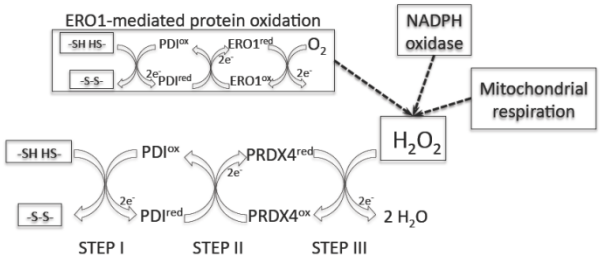
Protein folding in the endolasmic reticulum (ER) requires disulfide bond formation. The known enzymatic pathway to oxidative protein folding in the ER involves electron transfer from the client protein to protein disulfide isomerise (PDI) and from this one to endoplasmic reticulum oxidation 1 (ERO1) which reduces molecular oxygen and produces H2O2. Surprisingly, mammalian cells deficient in both ERO1 isoforms exhibit only a modest kinetic delay in disulfide bond formation and ERO1 knockout mice are viable and fertile [1]. To look for alternative pathways to oxidative protein folding in the ER a proteomic approach was used to identify proteins that accept electrons from a trapping mutant of PDI. Peroxiredoxin 4 (PRDX4) stood out in this proteomic analysis and its involvement in the oxidative folding in the ER was proved. Firstly, mouse embryo fibroblasts lacking ERO1 were intolerant to PRDX4 knockdown. Secondly, introduction of wild type mammalian PRDX4 into the ER of yeast (which lacks an endogenous ER PRDX) rescue the temperature-sensitive lethal phenotype of an ERO1 mutation. Thirdly, oxidative refolding of RNase A catalised by PDI and PRDX4 was achieved in vitro proving catalysis of protein disulfide bond formation by this novel pathway which couples oxidative protein folding in the ER to removal of toxic H2O2 formed by ERO1 (see figure bellow) [2].



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Molecular mechanisms controling chromosome segregation

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The molecular mechanisms ensuring accurate chromosome segregation during mitosis are critical to the conservation of euploidy in eucaryotic cells. Faithful chromosome segregation is essential to avoid the aneuploidy that leads to cancer and birth defects. Segregation is directed by the kinetochore, the protein complex that mediates attachment to the mitotic spindle and is required for chromosome movement during mitosis. One of our main interests is to establish if defective kinetochore components can generate aneuploidy. We are especially interested in the relationship between kinetochore structure and chromosome segregation.

We have recently identified and characterized the human Mob1-like proteins, a family of proteins required for proper cell division. We have found that Mob4 that downregulation of Mob4 causes severe defects in chromosome congression and segregation during mitosis. Interestingly, Mob4-depleted cells have reduced levels of Cenp-A, a histone H3 variant required for kinetochore formation, suggesting therefore that Mob4 regulates the assembly of the mitotic kinetochore.

Simultaneously, we have observed that reduced levels of Mob1 leads to a failure to complete cytokinesis in a way such that the cells stay connected by long intercellular bridges. Surprisingly, Mob1-depleted cells gain motility, moving rapidly and changing rapidly the direction of movement. These cells possess overstable microtubules with increased resistance to depolimerization, suggesting that the cytokinesis failure is caused by an overstabilization of the microtubules in the intercellular bridge.

In parallel studies, we found that Drosophila Mob1 and Mob4 mutants develop tumors indicating that Mob-like proteins are important players in the execution of cell division and that both Mob1 and Mob4 function as tumor supressores in high eukaryotes.

Signals in development and disease: Role of Cerl-2 in congenital heart malformations

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Mouse cerberus-like2 (*cerl-2*) is a Cerberus/Dan family member that is asymmetrically expressed on the right side of the mouse node. *cerl-2* encodes for a secreted protein that binds directly to *nodal* thus inhibiting its signaling pathway. *cerl-2* KO mice display multiple laterality defects including randomization of the L/R axis. However, we have found *cerl-2*-associated with cardiac defects that cannot be explained by laterality abnormalities like incomplete atrial and ventricular septation. Additionally was observed the increase of ventricular muscle, and to indicate that this special/singular phenotype is independent of LR establishment we have used the transgenic mouse line mlc1v-nLacZ24. In this way we propose that in addition to the previously described laterality-related defects, another distinct mechanism may contribute to the spectrum of complex cardiac defects in *cerl-2* KO mice. The molecular basis of vertebrate cardiogenesis is increasingly becoming understood. Research in this area will be an essential step, as the targets will be the most amenable sites of intervention, both in a therapeutic sense and for the purpose of prevention. Considering the high conservation of genetic pathways regulating cardiac development in species, the study of the human homolog genes involved in the nodal pathway is bringing us new data on Congenital Heart Disease (CHD) and on laterality defects. The human homolog of c*erl-2* (hCerl2 or DAND5) was also isolated, and maps to Chr. 19, region 19p13.13. We have started the molecular analysis of the human homolog of *cerl-2* and *nodal* in a set of CHD patients.

Chorea Huntington: A thousand changes due to a single mutation

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Chorea Huntington is a fatal neurodegenerative disease with late onset. Common symptoms are loss of motor control, cognitive decline, dementia and pathopsychological behaviour. The disease causing mutation – a CAG repeat expansion in a single gene named *huntingtin* (*htt*) - is well known and can be accurately diagnosed. Despite of intensive research, however, no cure for this devastating disease has been found yet.

Although Chorea Huntington is a classical Mendelian disease, various processes are involved molecular level ranging from transcriptional dys-regulation, protein aggregation, excitotoxicity and disruption of vesicular transport and mitochondrial metabolism. Furthermore, strong inter-individual variability in the disease progression suggests the existence of further biological modifiers which could provide novel therapeutic targets.

To consolidate the seemingly unrelated molecular changes observed and to detect novel disease modifiers, we constructed an *HTT*-focused protein interaction network. Introducing a novel multi-level prioritization strategy based on complementary information, we were able to identify a set of potential modifiers. One of the identified modifiers was subsequently experimentally validated as an important factor for aggregation, neurotoxicity and disease progression in HD models. Complementary to the focused analysis of the *HTT* and its interaction partners, we also applied a global approach to reveal connections between distinct disease-related processes.

Our study demonstrates that not only the elucidation of complex diseases but also of apparently ‘simple’ Mendelian diseases can tremendously profit from a network approach. The strategy proposed here can provide a general framework for the study of a wide class of human diseases.

The Neurobiology of Syntax: Recursion and Dynamical Systems

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The language faculty is a neurobiological system that provides humans with the capacity to understand, for all practical purposes, an unlimited set of sentences. At least since von Humboldt (1836), theoretical linguists have interpreted “practically unlimited” as meaning infinite and it is argued that this "linguistic infinity" necessitates a recursive syntactic rule set - a knowledge structure that, for example, allows humans to embed and understand phrases within phrases without limit. Hornstein argues that the language faculty must be simpler than previously thought. This is crucial, because ultimately, whatever syntactic operations linguists propose, these must be implementable in neural processing infrastructure. Thus, neurobiology puts hard constraints on the properties of the language faculty. We argue that the finiteness of neural systems, in terms of memory capacity and processing precision, is such a constraint. What are the implications of this for neurobiological models of syntax? First, we argue that it is not meaningful to separate "syntactic computation" from "processing memory"- or competence from performance in linguistic terms. Instead, the relevant fact is the human capacity to process bounded patterns of non-adjacent dependencies in language - there is a definite upper-bound on "distance" set by neurobiology. Second, we are free to choose any syntactic framework we prefer as long as this serves its purpose - for example, we may choose to capture non-adjacent dependency processing in bounded recursive formalisms. We illustrate this theoretical discussion with empirical results from behavioral, TMS, and FMRI investigations of Broca´s region in the context of implicit acquisition of simple artificial unification grammars.

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Expression and production of recombinant frutalin in different expression systems and evaluation of its biomedical applications

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Frutalin is the α-D-galactose-binding lectin expressed in breadfruit seeds (*Artocarpus incisa*). This lectin may be used in cancer diagnostics/therapeutics due to its potential ability to recognise specific carbohydrates expressed in cancer cells membranes and/or cells surface receptors. However, frutalin extraction from plant seeds is a time-consuming process and typically results in a heterogeneous mixture of different natural isoforms. To overcome these limitations, frutalin was cloned and expressed in *Pichia pastoris* [1] and *Escherichia coli* [2]. Recombinant frutalin was detected in cultures of these microorganisms by SDS-PAGE and Western blot analysis. The higher recombinant frutalin yield was obtained in the *P. pastoris* expression system (up to 20 mg/L). Molecular and biological differences were found between each recombinant and native frutalin. Potential biomedical applications for native frutalin and recombinant frutalin produced in *P. pastoris* were studied. Recombinant frutalin demonstrated higher capacity than native frutalin to differentiate malign from benign human prostate diseases by immunohistochemistry (with a significant positive statistical correlation, $P<0.00001$), in spite of its lower carbohydrate-binding affinity [3]. In addition, native and recombinant frutalin showed an identical magnitude of cytotoxicity on HeLa cervical cancer cells growth (IC_{50}=100 μg/mL, 24 h), by inducing cell apoptosis and inhibiting cell proliferation and migration. Interaction studies conducted by confocal microscopy showed that native and recombinant frutalin were internalised and targeted to HeLa cell's nucleus within 1 h of incubation. Therefore, frutalin with promising application in cancer diagnosis and therapy might be obtained from the recombinant *P. pastoris* expression system in alternative to its natural source.

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Bacterial Cellulose: production and applications

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Bacterial Celulose (BC) is a remarkable material, produced by the strictly aerobic bacteria *Gluconoacetobacter xylinum*. It is highly crystalline and pure cellulose, with outstanding mechanical properties, which may be obtained in a pre-defined shape. It absorbs a large amount of water, thus behaving like a highly stable and resistant hydrogel. In our group, we aim at developing applications in various fields, namely the production of biomedical devices, coatings for food products and composites with new properties.

The development of bioreactors for the large-scale production is a central topic, aiming at the affordable production of BC, allowing its use as a bulk material. In parallel, bioreactor design has been centered on the development BC structures with enhanced piezoelectricity. Such novel electro-active material could be explored as artificial muscles or in the tissue engineering of nerves.

Conductive polymeric composites have been receiving considerable attention because of their potential applications in electrodes, biosensors, batteries, antistatic coatings, gas sensors, membranes, light emitting diodes and notability in neuronal tissue engineering, robotics and biomedical actuators. The rationale is based on the putative modulatory effect of the electrical stimulation on cell attachment, proliferation, migration and differentiation. As such, research has been focused on the surface modification of BC by the covalent attachment of conductive polymers.

Applications in the biomedical field, as a scaffold for tissue engineering, requires the fine tuning of porosity and surface properties, allowing the cell migration and proliferation within the material. The development of artificial vascular prosthesis is one among the currently ongoing projects.

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Insights on Non-*Candida albicans Candida* species virulence factors

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Non-*Candida albicans Candida* (NCAC) species such as *Candida tropicalis, Candida parapsilosis* and *Candida glabrata* are responsible for 50 % of candidoses. It is important to highlight that candidose can be a superficial infection or a serious condition (e.g. systemic), which can lead to high morbidity and mortality. NCAC species virulence factors include: adhesion, biofilm formation and enzymes production. Therefore, our group focused its research on NCAC species virulence factors.

In the presence of urine NCAC species are able to colonize silicone (material commonly used on hospital catheters), with *C. parapsilosis* showing the lowest and *C. glabrata* the highest levels of adhesion. Biofilm formation studies demonstrated that NCAC species were able to form biofilm, although these were less extensive for *C. glabrata. C. glabrata* biofilm matrices' had high protein and carbohydrate, while *C. tropicalis* had low amounts of both components; but *C. parapsilosis* had high amount of carbohydrates and low protein.

The pathogenecity of NCAC species was evaluated by infecting human epithelium. *C. glabrata* adhered in higher extent but their influence on cell activity was less significant when comparing to other NCAC species. Moreover, *C. parapsilosis* revealed low invasiveness compared with *C. tropicalis* but both presented extensive damage after 24 h. RT-PCR results suggested that secreted aspartly proteinases were not involved on the invasion by *C. tropicalis* and *C. parapsilosis*, but indicated the role of these enzymes in tissue damage caused by *C. parapsilosis*.

The research performed by our group is a step forward on the development of new therapeutic approaches.

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Induction of hydrogen production affects micro and macro structure of granular sludge

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Mixed-culture dark fermentation is an environmentally friendly bio-hydrogen production process. In this work we study the potential for directing microbial anaerobic mixed communities towards improved hydrogen production. Strategies applied for promoting the selection of hydrogen-producing bacteria in anaerobic granules consisted of Heat treatment and chemical treatment with 2-bromo-ethane sulfonate (BES) and with BES+Chloroform. Three EGSB reactors, R_{Heat}, R_{BES} and $R_{BES+Chlo}$, where inoculated with each treated granules and fed with synthetic sugar-based wastewater. Hydrogen production was monitored. Morphological integrity and microbial diversity of the granules were studied using image analysis technique and 16S rRNA gene based techniques, respectively. Hydrogen production in R_{Heat} was below 300 $mLH_2L^{-1}d^{-1}$, with the exception of a single transient production of 1000 $mLH_2L^{-1}d^{-1}$, after decrease the HRT. In $R_{BES+Chlo}$ hydrogen production rate never exceeded 300 $mLH_2L^{-1}d^{-1}$. In this sludge, a physical deterioration of the granules was observed along with a decrease of their density and microbial diversity. In R_{BES}, a transient period of unstable H_2 production was observed but an additional pulse of BES triggered hydrogen production rate to an average value of 700 ± 200 $mLH_2L^{-1}d^{-1}$, which was kept for 30 days. This strategy did not affect significantly granules structure. Dominant bacterial ribotypes found in R_{BES} were closely related to *Clostridium* species and to uncultured microorganisms belonging to *Clostridiaceae* and *Ruminococcaceae*. This work demonstrates that different methods applied for directing granular sludge for hydrogen production can cause changes in the macro- and microstructure of granular sludge, which can be incompatible with the long-term operation of high-rate reactors.

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Strategies for the bioremediation of azo dyes containing wastewaters

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Azo dyes are an important class of wastewater pollutants resulted especially from textile industry. Biological treatment based on the anaerobic azo bound reductive cleavage, followed by a second step for the transformation of the resulted aromatic amines, seems promising. In our studies, the surface chemistry of a commercial activated carbon (AC) was selectively modified by chemical oxidation and thermal treatments and tested as a natural redox mediator on chemical and biological anaerobic azo dye reduction [1]. Batch experiments with 0.1 g L^{-1}AC demonstrated an increase of the first-order rates, up to 9-fold, as compared with assays without AC. Thermal treated samples gave better results due to their positively charged surface, favouring electrostatic attraction between the carbon and the anionic dyes tested. The low amount of AC used and the positive results demonstrated, constitutes a significant breakthrough in the field of redox mediated processes which will certainly open new perspectives for wastewater treatment processes. In order to investigate the fate of aromatic amines, two UASB reactors were operated under denitrifying conditions: R1 contained nitrate and R2 a nitrate and nitrite mixture as terminal electron acceptors [2]. The R1 results demonstrated that aniline could be degraded under denitrifying conditions while sulfanilic acid remains. The presence of nitrite in the influent of R2, caused a chemical reaction that led to immediate disappearance of both aromatic amines and the formation of an intense yellow solution. Based on the HPLC-MS analysis, the structures of possible products were proposed. Denitrification activity tests suggest some detoxification.

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Integrated system for macroalgae production and conversion into biogas

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Recently research on energy from macroalgae is being reconsidered driven by the following factors: fuel price increase, CO_2 mitigation policies and interest on renewable energy sources after the Kyoto protocol, and need for energy crops not competing with land for food production. However, the commercial expansion of this energy source is limited by its economic feasibility. In this presentation we analyse the development of integrated systems that promote synergies between macroalgae/biogas production and activities such as aquaculture and urban wastewater treatment. The recycling of nutrients and CO_2 by macroalgae can be an opportunity to reduce the biomass-biogas production cost. Other advantage is the proximity between biomass production, conversion into energy and its consumption, thus avoiding energy losses and pollution in transportation.

Experimental work is underway and includes batch tests to evaluate biodegradability of *Ulva* spp. and *Gracilaria* sp. and co-digestion of these macroalgae with sewage sludge from Beirolas wastewater treatment plant (WWTP). Furthermore, continuous co-digestion experiments are planned to be carried out in a 30 L experimental reactor to be placed in the same WWTP. The objectives are to reproduce sludge digestion in Beirolas WWTP and evaluate algae/sludge co-digestion performance.

Preliminary results indicate methane yields in accordance with similar batch experiments, whereby our CH_4 yields per volatile solids added range between 0.14-0.20 m^3 CH4 kg^{-1} VS_{added} against 0.16-0.27 m^3 CH4 kg^{-1} VS_{added} [1,2] depending on the algae species and pre-treatment. Overall, *Ulva* sp. shows a better performance over *Gracilaria* sp. as also confirmed in other experiments [1].

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Predicting SVI from activated sludge systems in different operating conditions through quantitative image analysis

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In wastewater treatment it is well documented that a variety of bulking phenomena, as well as other disturbances, can affect the normal behaviour of an activated sludge system, leading to lower treatment efficiency and biomass settleability. In the last few years, quantitative image analysis approaches, coupled to multivariate statistical analysis, have been increasingly used to clarify filamentous bulking detection and monitoring in activated sludge processes [1,2]. The present study focuses on predicting the Sludge Volume Index (SVI) for different types of conditions affecting an activated sludge system. To that effect, four experiments were conducted simulating filamentous bulking, zoogleal bulking, pin-point floc formation, and normal conditions. Alongside the SVI determination, the aggregated and filamentous biomass contents and morphology was studied, as well as the biomass Gram and viability status. Upon the determination of the image analysis data, regression analysis and partial least squares were used to reduce the dataset and model each studied condition. The obtained biomass contents and morphology data allowed establishing an SVI prediction ability presenting a regression value (R^2) of 0.8834, whereas the Gram and viability status data allowed for a regression value (R^2) of 0.793. It was also found that reasonable to good SVI prediction abilities were obtained using the biomass contents and morphology data, presenting correlation factors (R^2) of 0.7686 for the filamentous bulking conditions, 0.7831 for pin point floc formation, 0.9261 for zoogleal bulking and 0.8275 for normal conditions.

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New perspectives for methane production from oleate: bioaugmentation of anaerobic sludge with *Syntrophomonas zehnderi*

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Biogas production from waste lipids is a promising technology for sustainable energy production. In anaerobic bioreactors, lipids and long-chain fatty acids (LCFA) are easily removed from the liquid medium, mainly by adsorption. However, further LCFA degradation is rate-limiting and possible dependent on the development of syntrophic communities. Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA genes was used to follow the changes in bacterial communities during continuous and fed-batch reactors operation with oleate, an unsaturated LCFA. A specific dominant DGGE-band corresponding to bacteria deeply clustering with *Syntrophomonas zehnderi* (99% identity) was found in all the sludges that could degrade oleate, thus suggesting the involvement of this bacterium in unsaturated LCFA catabolism. Therefore, the potential of *S. zehnderi* as bioaugmenting strain for improving methane production from oleate was further studied in batch assays. Oleate was added to the medium at a final concentration of 1 mM and the assays were performed with and without the solid microcarrier sepiolite. Methane production was faster in the bioaugmented assays, and this effect was more pronounced in the presence of sepiolite. The positive effects of sepiolite can be related to a decrease in oleate toxicity towards the acetoclastic methanogens, or to an improvement of the syntrophic relationships. Bioaugmentation with *S. zehnderi* might be a suitable strategy for accelerating LCFA conversion to methane in anaerobic bioreactors, shortening the start-up period of high rate continuous processes or recover LCFA-inhibited sludges.

Acknowledgment: Financial support from Portuguese Science Foundation (FCT) through the PhD grant SFRH/BD/24256/2005.

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Bioleaching of hexavalent chromium from soils using *Acidithiobacillus thiooxidans*

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The continuous and growing degradation of the environment, due to several anthropogenic activities, is a main concern of the scientific community. Consequently, the development of low cost techniques to clean air, water and soils are under intense investigation. In this study, the focused problem is the soil contamination by hexavalent chromium, which is known for its several industrial applications - production of stainless steel, textile dyes, wood preservation and leather tanning - its high toxicity and mobility.

Bioleaching has been presented as a low cost effective technique to decontaminate soils polluted with heavy metals. Sulphur oxidizing bacteria, like *Acidithiobacillus thiooxidans*, were already applied with this technique as they produce sulphuric acid, lowering the pH and promoting the dissolution of heavy metals [1, 2]. On the other hand, it also known that polythionates, generated during the oxidation process, have high reducing power. Considering this information and since few studies have been made concerning the bioleaching of hexavalent chromium from soils, this work pretended to investigate this matter. Specifically, eighteen Erlenmeyers flasks (250 mL) with a working volume of 150 mL, containing 10% ($^{V}/_{V}$) of inoculum (*Acidithiobacillus thiooxidans* DSM 504), 90% ($^{V}/_{V}$) of growing medium (DSM 35) and 3% ($^{W}/_{V}$) of contaminated soil were agitated in a rotary shaker, at 150 rpm, for 70 days. Also three controls were undertaken by sterilizing the soil and/or suppressing the inoculum. Two levels of soil contamination were evaluated within this work.

This study presented bioleaching as a competitive technique in soil cleaning, as it is efficient and inexpensive.

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Protozoa grazing evaluation: a novel way to assess wastewater treatment performance?

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Protozoa are recognized as essential to reach high-standard performances in wastewater treatment plants (WWTP), namely activated-sludge, by drastically reducing the number of dispersed bacteria and therefore the turbidity of the final effluent. Moreover, protozoa are sensitive to environmental variations and changes in these populations are known to affect the whole food-web, thus affecting the performance of the wastewater treatment plant.

The analysis of the protozoa populations is currently used to assess the performance of WWTP and the impact of protozoa grazing on the survival of particular groups of bacteria has been studied. Nevertheless, no studies exist on the possibility of simply evaluating the grazing rate of protozoa to assess the ecosystem health and therefore the WWTP performance.

The results obtained in the present study suggest that protozoa grazing reflects the health of the whole community inhabiting the aeration tank of WWTP and therefore can be used to evaluate the performance of the treatment system. Grazing was assessed by determining the ingestion of GFP (Green Fluorescent Protein) E. coli by the sessile ciliate Epistylis sp. using fluorescence microscopy. The samples were also inspected to allow for the determination of the Sludge Biotic Index (SBI), routinely used to evaluate WWTP performance.

The grazing rate clearly and significantly reflected the IBL evaluation. The study stands for the possibility of using grazing assessment as an alternative to the highly-expertise skills demanding SBI. More studies will be needed to investigate the possibility of using other species and/or genera.

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Denitrification and Ozonation Processes for Mature Landfill Leachate Treatment

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The current problematic of leachate management in Portugal is intimately connected with the inefficiency of the systems in operation, with subsequent discharge into sewers and water streams of effluents still with high levels of contamination. More specifically, many leachate treatment or pre-treatment plants have been experiencing difficulties in the removal of nitrate (NO_3^-).

The main objective of this work was to evaluate the removal of nitrate from a mature landfill leachate with high NO_3^- load by denitrification in an anoxic rotating biological contactor (RBC).

The anoxic lab-scale reactor was inoculated with acclimatized activated sludge and operated in a continuous mode, with a hydraulic retention time of 10 h.

Under a phosphorus-phosphate concentration of 10 mg $P\text{-}PO_4^{3-}\cdot L^{-1}$ and nitrogen-nitrate concentrations above 530 mg $N\text{-}NO_3^-\cdot L^{-1}$ the reactor achieved nitrogen-nitrate removal efficiencies close to 100%, without nitrite or nitrous oxide accumulation. Although the reactor presented a very good denitrification performance, the effluent carbon concentration was still above the legal discharge value.

In order to increase the biodegradability of the leachate recalcitrant carbon load, a pre-ozonation was further investigated. The pre-ozonation led to a total organic carbon (TOC) removal of 28%. The sequence of treatments, leachate ozonation followed by RBC denitrification did not affect the denitrification efficiency. In fact, it was possible to attain a denitrification rate of 123 mg $N\text{-}NO_3^-\cdot L^{-1}\cdot h^{-1}$. The moderate decrease in the carbon load of the final effluent indicated that some recalcitrant compounds were still present after ozonation. The anoxic RBC showed to be a promising technology for removing nitrate from landfill leachate.

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Uptake, equilibrium and kinetics studies for the adsorption of 3- pentanone onto four different clays

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The removal of organic solvents from contaminated systems is a problem of particular concern. In fact, these substances are subject to increasing severe environmental constraints because of their direct danger to and impact on health and the environment.

This work aims the development of an environmental technology applicable to the treatment of aqueous solutions contaminated with low concentrations of solvents. Due to its widespread use, special attention will be given to the solvent diethylketone. Batch studies were made aiming to investigate the adsorption behaviour of four different clays for the treatment of diethylketone aqueous solutions. The effect of the mass of adsorbent was studied. Experimental equilibrium results were analysed using the Dubinin-Radushkevich and Sips adsorption isotherms and kinetic data were analysed by pseudo-first and pseudo-second order models.

Almost complete removal of diethylketone was achieved for all the clays, with values of removal percentage around 97%. The adsorption performance, in terms of uptake, is vermiculite > sepiolite > kaolinite > bentonite, for the mass of 0.1 g in 150 mL of 800 mg/L of ketone solution.

The best isotherm fit for this solvent by bentonite and kaolinite clays was obtained with the Sips model while the Dubinin-Raduskevich model was the best option for sepiolite and vermiculite. Kinetic data were described by pseudo-first and pseudo-second order kinetics model and the best fit was obtained for the pseudo-first order model that assumes that the interaction rate is limited only by one process or mechanism on a single class of sorbing sites and that all sites are of time dependent.

The study showed that the clays tested are very promising for the removal of solvents from effluents.

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Poly (ε-caprolactone) as biofilm support and carbonsource for groundwater denitrification

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Groundwater is widely used as a drinking water source in most countries of the world. However, groundwater nitrate contamination has steadily been increasing over the years as a consequence of anthropogenic activities. Elevated nitrate concentrations in drinking water sources can cause diseases such as methahemoglobinemia and stomach cancer.

Traditional physical and chemical processes such as reverse osmosis, ion exchange, electrodialysis and chemical denitrification have been used for nitrate removal from water but despite their effectiveness, they are very expensive. Therefore, it is crucial to explore alternative strategies to remove nitrate of groundwater.

The use of biological denitrification to convert nitrate to harmless N_2 gas and nitrous oxide represent a good alternative treatment process for the remediation of groundwater contaminated with nitrate due to the elevated specificity of denitrifying bacteria, low cost and high denitrification efficiency. Typically, contaminated groundwater with nitrate is severely limited in organic carbon and the addition of an external soluble carbon source (e.g. acetic acid, sucrose, ethanol and methanol) is the usual procedure to achieve nitrogen removal. Nevertheless, the costs associated and the risk of additional contamination of the environment involved in this procedure demand the development of innovative treatment strategies. In this context, the application of biodegradable polymers (solid carbon sources) has been gaining importance in groundwater denitrification process. Solid carbon sources serve not only as sources of reducing power for denitrification but also as solid matrices for biofilms development. Moreover, in contrast to conventional processes, the use of this kind of carbon sources has no potential risks of release of excess dissolved organic carbon with the resultant deterioration of water quality.

The aim of the present work was to investigate the feasibility and efficiency of nitrate removal from groundwater by biological denitrification in column laboratory reactors packed with supports of poly (ε-caprolactone) (PCL). The maximum denitrification rate attained with PCL was 4.38 mg/L.h $N\text{-}NO^-_3$ at velocity of 0.08 m/h, at 20 °C and pH 7.0.

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Bioconversion of azo dyes in activated sludge sequencing-batch bioreactors

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An overview of the work carried out at CEBQ-IST (ENVERG group) on the bioconversion of azo dyes in activated sludge sequencing-batch bioreactors will be presented. Studies on the relevance of operational parameter changes have been done, covering sludge age, hydraulic retention time, anoxic-oxic stage scheduling and temperature, as well as changes in the nature of the main carbon source and electron donor provided for biomass growth and dye reduction. Results on dye decolourisation kinetics and yields and on the fate of the primary metabolites (aromatic amines) will be presented, together with those of a few observations on the dynamics of the microbial populations. This work allowed the identification of key possibilities and problems involved in the treatment of dye containing wastewaters in activated sludge bioreactors. Further studies involving changes in the electron acceptors or transporters available to the dye-transforming biomass are also included in this review, highlighting the importance of redox pathway versatility in broadening the range of biotransformed azo dye types. Recent studies on the feasibility of using spectrophotometry in the UV-visible and NIR ranges for real-time process supervision in sequencing-batch bioreactors bioconverting azo dyes will also be presented. These include investigations into the use of spectra to rapidly predict dye and metabolite concentrations in the medium and into the possibility of spectra-based process supervision.

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Assessment of the biogenic content of Solid Recovered Fuels by chemical dissolution and radiometric methods

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Biogenic fraction quantification of solid recovered fuel (SRF) is of great importance in order to evaluate its non-fossil and renewable potential as an alternative fuel. It corresponds to the fraction of material that is produced by living organisms but not fossilized or derived from fossil resources. Therefore, it is a “classification” aspect which needs to be assessed rather than a chemical or a physical property.

Selective dissolution (SD) is a fast and cost effective method that takes advantage of the carbon reactive groups found in biogenic materials, which are readily degradable in acidic media, by hydrogen peroxide. On the other hand, some non-biogenic waste materials, e.g., soft polyurethanes, degradable plastics, etc. react as well. So, the way interfering materials alter the biomass content when SD is used needs explanation. A linear interference between soft polyurethanes (PUR) and SRF biogenic content was noticed. Deviations of 1.25% to 3.08% for 5% and 10% of PUR incorporation into SRF were estimated. The SD seems to be unaffected by baby diapers components.

The Radiocarbon (^{14}C) method is a future alternative for SRF biogenic content assessment. In contrast with fossil resources, natural materials are expected to have ^{14}C, an unstable carbon isotope which tends to decay over time. By liquid scintillation counting (LSC), the ratio of ^{14}C is being explored. The initial CO_2 capture revealed as a crucial step for effective LSC reading. On the other hand, counting step could take more than 8 hours to reduce the associated error.

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Production of Homo-, Co- and Ter- Bacterial Polyesters with Waste Glycerol as Major Carbon Source

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Polyhydroxyalkanoates (PHAs) are polyesters synthesized under unbalanced growth by many bacteria as intracellular carbon and energy storage compounds. A wide spectrum of biocompatible polymers with varying properties can be obtained, suitable for multiple applications in fields such as medicine and agriculture. Nowadays, a few more than a dozen companies produce microbial PHAs at industrial scale and, apparently, all of them use noble carbon sources. Economical evaluation studies led to the conclusion that 48 % of the production costs is ascribed to the raw materials, the C source accounting for 70-80 % of the total cost [1]. Aiming at reducing these costs, the development of high productivity cultivation processes based on waste glycerol (GRP), a by-product of the biodiesel industry, is being addressed. We utilized a *Cupriavidus necator* strain to produce the homopolymer P(3HB), the copolymer P(3HB-co-4HB) and the terpolymer P(3HB-4HB-3HV) using, in all cases, GRP as the major C source. Fed-batch high cell density culture strategies were developed in 2L STRs. P(3HB) productivities between 0.84 and 1.1 $g_{PHB}.L^{-1}.h^{-1}$ were achieved [2]. P(3HB-4HB) copolymers with various 4HB % were produced using gamma-butyrolactone as co-substrate. The highest 4HB% attained was 12.3 % with a productivity of 0.79 $g_{PHA}.L^{-1}.h^{-1}$. The average weight molecular weight of the copolymer ranged from 2.5×10^5 to 1.4×10^6 Da. The productivity in terpolymer was 0.48 $g_{PHA}.L^{-1}.h^{-1}$. The 4HB% and the 3HV% varied between 13 and 26, and 8 and 4%, respectively, during the accumulation period. The thermal properties, determined at the CEIB, Université de Liège, considerably changed with the monomer composition and percentage incorporation, the MW and the polydispersity index.

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Applying enzymatics, transcriptomics and proteomics to phytoremediation

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The role of *Phragmites' sp.* in a vertical flow constructed wetland has been studied using an azo dye, Acid Orange 7 (AO7), as an organic pollutant. Despite the efficiency of the system[1], the mechanisms used by the plant cells to perceive, transduce and respond to the presence of AO7 are yet to be unveiled, likewise, the functional assignment of the major enzymes/proteins involved in the process. Enzymatic, transcriptomic and proteomic analysis have been applied to study phytoremediation.

Enzymatic activities of reactive oxygen species (ROS) scavenging enzymes, such as superoxide dismutase (SOD), peroxidase, catalase (CAT) and ascorbate peroxidase were found to increased significantly from 15 min to 1 hour after *Phragmites* were exposed to AO7 (400 mg/L) suggesting a response to the chemical stress. The enhancement of glutathione *S*-transferase suggests the activation of AO7 plant cell conjugation.

An increased transcription level of genes encoding the enzymes SOD, CAT and glutathione peroxidase involved in oxidative stress response was observed, establishing a correlation between AO7 exposure and the plants reaction by producing ROS[2]. This work was developed as collaboration between ENVERG-BERG.

Currently, a quantitative proteomic analysis is underway to enable the comparison of proteins from *Phragmites sp.* fed with water and after different AO7 contact times. Preliminary results show that the abundance of 249 proteins increased, whereas 185 decreased. In both cases, after 24 hours protein abundance returned to unstressed plant levels. This work is being carried out as collaboration between ENVERG-BSRG.

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Environmental genomics: unveiling the mechanisms of toxicity and resistance to the herbicide 2,4-D

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Environmental genomics, the genome-wide analysis of cellular adaptive responses to chemicals with impact in the environment, is a key tool in the understanding of the mechanisms of toxicity and resistance to these compounds [1]. This knowledge is crucial to allow early toxicological prediction and the design of strategies to deal with pesticide-resistant weeds and fungi.

In this study, the model eukaryote *Saccharomyces cerevisiae* was used to unveil the mechanisms of toxicity of, and resistance to, the widely used herbicide 2,4-D. Transcriptomics [2] and expression proteomics [3] were used to assess the global yeast response to inhibitory concentrations of this herbicide and guided more detailed studies [4, 5, 6] into the mechanisms that allow yeast cells to thrive in this hostile environment. Particular emphasis was given to the role of multidrug resistance (MDR) transporters, since their characterization using functional genomics tools has been one of our goals since the yeast genome sequence was made available [7]. Tpo1, a Drug H^+-Antiporter of the Major Facilitator Superfamily (MFS), and Pdr5, an ATP-Binding Cassette (ABC) drug efflux pump, were shown to confer resistance to 2,4-D [4], playing a role in reducing the intracellular accumulation of the herbicide [6]. Furthermore, the effect of predicted Tpo1 homologues from the plant model *Arabidopsis thaliana* in herbicide stress resistance was analysed, revealing the existence of a plant MFS transporter which confers resistance to 2,4-D and other environmental stresses [6].

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The effect of HZSM-5 zeolite acidity on the catalytic degradation of high-density polyethylene

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In view of their versatility and relatively low cost, the consumption of plastics, and the consequent amount of plastic waste, are growing year after year. Polymer waste can cause serious environmental problems and they are usually dumped or incinerated, and their potential energy content is wasted. Therefore, conversion of waste plastics to useful hydrocarbons in the liquid fuels range [1] by catalytic degradation is a promising alternative with a great potential for energy saving as well as constituting a solution to the pollution problem. Resorting to catalysts, this process simultaneously provides a useful destination for plastic waste, saves precious landfill capacity, partially replaces the consumption of petroleum-based fuels, and reduces CO_2 emissions.

Zeolitic catalysts, due to their characteristic strong acidity, have the ability to cleave carbon-carbon bonds which, together with their widespread use in the hydrocarbon processing industry, makes them a distinct possibility to promote the catalytic degradation of plastics.

In this way, we have investigated the effect of the acidity of HZSM-5 zeolites on the catalytic degradation of high-density polyethylene (HDPE). The acidity of the zeolite was modified by ion exchange with sodium. The results show that, strong acidity reduces the degradation temperature leading to reduction of the energy consumed in the process.

Additionally, the product distribution can be tuned by changing the catalyst acidity and operating conditions.

The simultaneous use of the signals from the thermogravimetric (TG) and differential scanning calorimetric (DSC) allowed the development of a kinetic model that permits the analysis of polyethylene pyrolysis [2].

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Green processing of chitin porous structures combining ionic liquids and supercritical CO_2

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The application of green chemistry principles in the processing of materials can provide a significant advance in the development of a more environmentally benign approach towards the fabrication of biomaterials with well-defined architectures for advanced technologies [1]. This work intends to apply the green chemistry principles in the development of chitin-based porous materials through combination of green solvents, such as ionic liquids (ILs) in dissolution of chitin together with the use of a clean and environmentally friendly technology, namely supercritical fluid technology (SCF) [2]. The unique solvent tuneability of SCF, from gas-like to liquid-like properties, offers the possibility of precise control over the processing conditions, which can be adapted in many ways for the extraction of ILs. With this in mind, chitin was dissolved in 1-butyl-3-methylimidazolium acetate, ([bmim][Ac]), followed by regeneration of polymer in ethanol in specific moulds. The [bmim][Ac] removal was performed using soxhlet extraction and successive steps of extraction with dioxide carbon/ethanol (CO_2/etOH) ratios at near critical conditions. Later on, the samples were dried with CO_2 under supercritical fluid conditions, and characterized using different techniques. The developed chitin porous structures can be classified as mesoporous materials, with low density and high porosity. Most of these features were associated to dissolution and regeneration of chitin in ethanol and, to IL removal process applied. The findings demonstrated that the strategy applied was a convenient way to prepare chitin porous structures. The prepared matrices are therefore potential candidates for various relevant biomedical applications.

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Valorization of marine resources: unraveling high-potential materials

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Marine organisms are constituted by materials with a vast range of properties that may justify their application within the biomedical field. Moreover, assuring the sustainable exploitation of natural marine resources, the valorization of residues from marine origin, like those obtained from food processing, constitutes a highly interesting platform for development of novel biomaterials, with both economic and environmental benefits [1]. In this perspective, this research group is coordinating a project on valorization of marine resources and residues – IBEROMARE – with a strategy based in three stages. First, marine resources with higher potential for valorisation are identified. Then, strategies for the valorisation of those raw materials are proposed and further studied, considering the extraction of valuable compounds and their further use for different scientific and industrial fields. Finally, it is envisaged the collaboration with industrial partners in order to assess the applicability of the proposed strategies as technologies for valorisation of residues or as technologies to achieve market relevant applications.

In particular, a biorefinery-like concept is being developed based on crab shells, in order to obtain added-value from all its main constituents. In a biomedical focused approach, it was achieved the isolation and characterization of ulvan [2] and chitosan from green algae and pens of cephalopods, respectively. After demonstrating their non-cytotoxic behaviour, those materials are currently being studied as biomaterials for biomedical applications, namely, tissue engineering and regenerative medicine. Moreover, extraction of BMPs from fish bones is being assessed, for further use in bone tissue repair and regeneration, exploring a less expensive alternative to BMP production by recombinant technology.

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AGRO-FOOD BIOTECHNOLOGY

Oral Communications

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Influence of electric field in the physical and transport properties of chitosan coatings

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Edible films and coatings can provide additional protection for food, while being a fully biodegradable, environmentally friendly packaging system. The aim of this work was to determine the effect of field strength on functional properties of chitosan coatings. Different field strengths were applied during the preparation of the film forming solution, films were cast and, for each electric treatment, the water vapor, O2 and CO2 permeabilities of the films were determined, together with their solubility in water and mechanical properties. The films were also analysed using scanning electron microscopy (SEM) and X-ray diffractometry (XRD).

The results showed that the electric field has statistically significant effects on films' transport properties (which e.g. for water vapour permeability, varied from 0.3228 to 0.2667 $(g.(m.day.atm)^{-1})$) and structure, a positive correlation having been found between the water vapor, O_2 and CO_2 permeability coefficients and the applied field strength.

XRD analyses indicated that electrically treated chitosan films exhibited a more ordered structure and a clearly higher crystallinity when compared with non-treated films, thus displaying significant effects on the value of the crystallinity index (CI). SEM micrographs evidenced that the surface morphology of chitosan films was influenced by the electric field. In fact, the electric field treatment led to a structure with more regular layers. The application of the electric field to chitosan film-forming solutions resulted in an increase of the tensile strength (ca. 9 %) and elongation-at-break (ca. 18 %) of the corresponding chitosan films. The reported results demonstrate that the application of an electric field to film-forming solutions of chitosan is an interesting instrument to tailor relevant properties of the films or coatings produced from them.

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Overview of strategies for edible coating formulations - applications on food quality and safety

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Edible coatings can be used to improve shelf-life and food quality by providing good and selective barriers to moisture transfer, oxygen uptake, better visual aspect, and reduction of microbiological contamination.

The effectiveness of edible coatings depends, in a first stage, on the control of the wettability of the coating in order to ensure a uniformly coated surface, and in a second stage on other factors (gases permeability; opacity; mechanical properties) that can also affect the effectiveness of the coating and that strongly depend on the food surface that will be coated. A methodology which is primarily based on wettability should therefore answer adequately the challenge of optimizing edible coating formulations. Such methodology has been developed by our group [1, 2] and it has been shown that it can be used for fruits, vegetables and cheese. In all the cases different formulations of edible coatings (polysaccharides, proteins, plasticizers, surfactants and lipids) were evaluated through the measurement of the wettability on foods. Then the coating compositions with the best values of wettability were selected and the barrier, colour and mechanical properties were evaluated in order to select a unique coating composition. The results have shown that the final composition allows in all cases a good coating performance with a good coating adhesion.

This methodology allows testing a great number of formulations and its application has generated an important amount of data on the use of edible coatings on several foods, particularly in order to extend their shelf-life and quality.

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Effects of moderate electric fields on aggregation of whey protein solutions and properties of edible films made thereof

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Thermal processing may result in disruption of the native conformation of whey proteins, thus affecting their structure and functional properties. The aim of this work was to evaluate the effects of moderate electric fields on thermal aggregation of whey proteins and its subsequent effects on properties of edible films made from ohmic heated whey protein film forming solutions. Thermal aggregation of whey protein isolate was studied at 85 ºC up to 30 min through ohmic heating, under the presence of moderate electric fields ranging from 4 to 20 V/cm; this treatment was compared with conventional heating under identical temperature profiles. Results show that whey protein aggregation (measured by dynamic light scattering in terms of aggregate size) was found to decrease with the increase of electric field applied during ohmic heating; a maximum increase in whey protein aggregation of 45 nm was observed for treatments at 18 V/cm, while treatments at 4 V/cm and 0 V/cm (conventional heating) produced a maximum increase of 70 nm and 75 nm, respectively. Edible films prepared from ohmic heated film forming solutions present a decrease of about 10 %, when compared with films produced through conventional heating, for water vapor permeability. The presence of moderate electric fields during heating, apparently influences the denaturation and aggregation of whey proteins thus modifying the mechanisms of protein interactions in films formed from whey protein film forming solutions. Ohmic heating presents itself as a novel method for production of protein films.

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A decade of mycotoxin research at the Institute of Biotechnology and Bioengineering

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Filamentous fungi are fundamental to applied microbiology. An area of increasing concern is contamination of foodstuff with toxins from these fungi, which are referred to as mycotoxins. The mycotoxin secondary metabolites cause disease in animals and humans and, for example, aflatoxins have been responsible recently for fatal human outbreaks in Kenya. Our researchers have been involved in research on mycotoxins and the work has grown exponentially in the previous decade. Mycotoxin problems commence locally and extend globally: Bottled water and wines from the North of Portugal have been studied with a view to control fungi and mycotoxins. Now we are expanded into considering the implications of climate change on mycotoxins on a worldwide basis. The use of mycotoxins and fungi as bioweapons in our increasingly security-conscious society, has been discussed by our researchers. Surprisingly, the fungi that produce mycotoxins are required to be characterized using novel methods as conventional procedures often prove to be inadequate. Undertaking this task is a primary objective of our research. Novel fungal identification schemes have been devised and issues of whether isolated taxa truly represent those in the environment have been addressed, with ramifications relating to optimal control. Current projects include the health and quality risks from fungal contamination of grape products (e.g. wine), apples, cheese, chilies, nuts and corn: Fungi in drinking water are a particular concern. *Aspergillus ochraceus*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium expansum* and *Fusarium graminearum* especially are relevant to our work: Associated mycotoxins are ochratoxin A, aflatoxins, patulin, citrinin and zearalenone. Furthermore, we study the volatile earthy off-aromas in wine caused by the interactions of the fungi *Botrytis cinerea* and *P. expansum*. The analytical techniques employed throughout our work include PCR, HPLC, GC-MS and MALDITOF MS for strain characterization and analysis of commodities. Critical assessments of these methods have been performed and potential problems discovered with diagnostic PCR equally apply to bacteria. A key objective is international collaboration with other researchers. Our strains are well characterized for toxicity and are kept under optimal preservation techniques. This presentation will describe the progress made by this innovative laboratory over the previous 10 years, while looking forward to future progress.

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The use of bacteriophages to control biofilms

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After several years of abandonment, the use of bacteriophages (phages) for killing bacteria has withdrawn recent attention and appraisal. This has led to a vast phage research, in varied fields, with impressive outcomes. Despite this enthusiasm, there is a lack of research concerning phage utilization to reduce bacteria living on surfaces in a lifeform known as biofilms.

This work explores the potential of newly isolated phages in controlling bacteria present in single and dual species biofilms. Gram-negative *Pseudomonas fluorescens* and Gram-positive *Staphylococcus lentus*, widespread inhabitants of dairy plant surfaces and products were the studied bacterial hosts.

Two broad host range phages belonging to the *Podoviridae* family, phiIBB-PF7A for *P. fluorescens* and phiIBB-SL58B for *S. lentus* were selected for the experiments. Both phages were efficient towards single species biofilms of each host, even against 7 days old biofilms. Furthermore, phage phiIBB-PF7A showed ability to infect and control cells with two distinct morphologies (rod and elongated) resulting in different numbers of progeny phages released after infections of these different hosts. Although phages need actively reproducing host, we obtained good destruction of cells living under severe starvation conditions and of cells in the stationary growth phase [1].

Dual species biofilms were challenged using two approaches: i) a phage cocktail and ii) a single phage for the less predominant species. The cocktail with phages for each of the dual species biofilms host decreased efficiently not only the cell number in the biofilm, but also the cells which were released to the planktonic phase while, on the other hands, the use of a single phage caused the release of the non-susceptible species to the planktonic phase [2].

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Cross-contamination in food-contacting surfaces: novel approaches to control food-borne pathogens

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Food-contacting surfaces can be easily contaminated with pathogenic bacteria during food processing being a source of contamination. This phenomenon, known as cross-contamination, is a common factor responsible for many foodborne outbreaks that leads to wide economic loss and has a strong impact on public health worldwide. Several approaches have been used in order to minimize this problem, being the modification of food-contacting surfaces (incorporation of antimicrobial compounds) and chemical disinfection (development of effective sanitizers) the most common.

Accordingly, the ability of adhesion and biofilm formation by *Salmonella* Enteritidis on kitchen bench stones and on stones with Microban® incorporated was assessed. The results revealed that all stones tested are prone to bacterial adhesion and no considerable effect of triclosan was observed in both silestones. These results points to the need of using sanitizers. In this context, the susceptibility of *L. monocytogenes* and *S. enterica* biofilms to four disinfectants – sodium hypochlorite, benzalkonium chloride, hydrogen peroxide and triclosan was studied. It was observed that biofilms from both bacterial species were more susceptible to sodium hypochlorite than to any other disinfectant. However, chemical disinfection can lead to the acquisition of bacterial resistance since it was observed that disinfection survival cells seem to develop a stress response and/or become more virulent, which may compromise food safety and represents a potentially increased risk for public health. In order to overcome these disadvantageous, the potential of photocatalytic disinfection (TiO_2-coated surfaces) was also assessed. Although it has been revealing as a promising alternative, some improvements have yet to be made.

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Friendly technologies in agro-food and quality control

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The agro-food biotechnology group of ENVERG aims to use environmentally clean processing technologies in the preparation of value-added food and cosmetics and/or their ingredients, as well as the evaluation and modelling of the methodologies and products obtained. The group has been also successful in the area of minimal processing of fruit.

The major achievements are: i) Modelling of supercritical fluid extraction (SFE). ii) Production of essential oil from *Myrtus communis*, and extracts from the deodorised myrtle, obtained by using solvent extraction and SFE[3-5]. iii) Production of two products from SFE carob pulp – the extract, rich in phenolic compounds (~16g/kg), with antioxidant and anticancer activities; and a ligno-cellulose co-product (solid residue), rich in insoluble fibre (47-62%), and soluble fibre (5-10%), and also in phenolic compounds (~30g/kg), with high functional activity [1]. iv) Environmentally friendly minimal processing techniques to successfully prolong the high quality life period (fresh-like) of cut *Rocha* pear, which is the most exported fruit (as such) from Portugal. Values obtained indicate that a minimally processed product would withstand, under refrigeration, the time period necessary for its export under this value added form [2]. v) Customer satisfaction survey - discovering a possible opportunity of product innovation CEM methodology that will make the product more enjoyable for clients reflecting also the customers' concerns like the price, the sustainability of the product and the proximity of the product to the people. vi) Quality management methods – characterization of process variables allows controlling the product along the production process. The goal was the process optimization through variability analysis.

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New insights in the molecular events underlying *Medicago-Sinorhizobium* biological nitrogen fixation symbiosis

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Legume plants have developed an intimate association with nitrogen-fixing rhizobial bacteria that provide plants with a reliable nitrogen source. Central to this plant-bacteria interaction is the formation of root nodules. Several symbiosis genetic determinants have been characterized, one of them being the outer membrane protein TolC from *Sinorhizobium meliloti*. TolC is required for establishing symbiosis with the plant *Medicago sativa* as well as for protein and exopolysaccharide secretion and protection against osmotic and oxidative stresses [1]. As a step toward understanding the physiology of the *S. meliloti* 1021 *tolC* mutant, its transcriptional profile was determined [2]. The significantly induced genes suggest the activation of cytoplasmic and extracytoplasmic stress responses. These stress conditions are most probably caused by protein accumulation both in the cytoplasm and periplasm and by oxidative stress. The activation of an oxidative stress response in the *tolC* mutant was confirmed by the increased levels of the enzymatic activities of catalase, superoxide dismutase and glutathione reductase. The absence of a functional TolC caused a decreased in the expression of genes encoding products mainly involved in nitrogen metabolism, transport and cell division. Two genes strongly induced in the *tolC* mutant were homologues to CpxAR two-component regulatory system. Although Cpx proteins were initially known by responding to adaptation to cell envelope damage, recent studies link Cpx proteins to adhesion processes. Since *cpx* homologues have a crucial role mediating adhesion in other bacteria, it is our aim to assess the involvement of this two-component regulator in *Sinorhizobium* root-adhesion and symbiosis.

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Chemotypes in *Thymus caespititius*: a molecular biology approach on TPS genes

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The use of molecular markers such as RAPD and ISSR has been extremely important to characterize plant genetic diversity. However their application to understand the chemical polymorphism of aromatic plants has been of limited value for several tested species. The studies performed so far with species from the Lamiaceae [1], Apiaceae and Pittosporaceae, using this molecular approach did not show any correlation between molecular and chemical data. In view of this, we've been developing more targeted approaches namely the characterization of genes involved in essential oil production.

Thymus caespititius (Lamiaceae) appears to be a good model plant for this kind of studies as it possess four defined chemotypes (thymol, carvacrol, α-terpineol and sabinene), besides some mixed chemotypes. Our results on the characterization of terpene synthase genes (TPS), using both gDNA and cDNA from plants belonging to specific chemotypes, include the γ-terpinene synthase and sabinene synthase genes. These results have revealed a gene structure similar to TPS-b class described previously [2], with 7 exons and 6 introns. A cDNA library using RT-PCR is in construction and expression analysis using different plant tissues are in progress in order to bring light to the relationship between gene expression and the defined chemotypes.

Acknowledgements: Partially funded by FCT under research contract PTDC/AGR-GPL/101334/2008.

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Whole blood transcriptional profiling in ankylosing spondylitis identifies novel putative candidate genes for both the inflammatory and tissue-destructive aspects of the disease

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Background: The aetiopathology of ankylosing spondylitis (AS) is poorly understood. Transcriptional profiling generates a “snapshot” of the sampled cells activity and thus can provide insights into the molecular processes driving the disease process. Several recent studies have defined transcriptional profiles generated from peripheral blood mononuclear cells (PBMCs) however PBMC isolation is not viable in multicentre studies and limits the viability of such an approach. An alternate approach is to use whole blood samples collected using PAXgene technology which preserves integrity of the RNA.

Objective: We undertook a whole-genome microarray approach to identify candidate genes associated with AS and validated these gene-expression changes in a larger sample cohort.

Methods: 18 active AS patients, classified according to the New York criteria. and 18 gender- and age-matched controls were profiled using Illumina HT-12 Whole-Genome Expression BeadChips which carry cDNAs for 48000 genes and transcripts. Class comparison analysis identified a number of differentially expressed candidate genes. These candidate genes were then validated in a larger cohort using qPCR-based TaqMan Low Density Arrays (TLDAs).

Results: 239 probes corresponding to 221 genes were identified as being significantly different between patients and controls with a p-value <0.0005 (80% confidence level of false discovery rate). Forty eight genes were then selected for validation studies, using the TLDAs. Thirteen of these genes were validated in the second patient cohort with 12 down-regulated 1.3-2-fold and only 1 upregulated (1.6-fold). A number of these genes, have well-documented inflammatory roles with PTPN1 & DOCK10 both involved in mediating IL4

action. Of specific interest to AS progression are the established roles of SPOCK2 (osteonectin) and EP300 in cartilage. DNMT1 and EP300 both mediate STAT3 functionality which has also been associated in genetic studies with AS.

Conclusion: We have validated a gene expression signature for AS from whole blood and identified strong candidate genes that may play roles in both the inflammatory and joint destruction aspects of the disease.

Adaptive response and tolerance to acetic acid stress in yeast: a genome-wide view

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Acetic acid is a byproduct of alcoholic fermentation by *Saccharomyces cerevisiae* and its accumulation in the medium at low pH may contribute to fermentation arrest. Acetic acid is also present in lignocellulosic hydrolysates. Since yeast-based production processes from lignocellulosic feedstocks are anticipated within the next years (the first products will be biofuels), a better understanding and improvement of yeast tolerance to acetic acid is essential. Acetic acid is also widely used as a food preservative but spoilage yeast strains highly tolerant to this weak acid may limit its use in the Food Industry. This presentation will focus on the results obtained by our research group on the adaptive responses and resistance mechanisms of yeast cells to acetic acid stress, exploring genome-wide approaches [1]. This knowledge is essential for the engineering of more robust industrial yeast strains and selection of optimal fermentation conditions and to guide more efficient food preservation strategies.

Based on a transcriptomic analysis, the transcription factor (TF) Haa1p, previously associated to yeast response and resistance to acetic acid [2], was found to be the main player in the control of the alteration of yeast genomic expression program in response to this stress [3]. Quantitative proteomics was also explored to further understand the role of the Haa1p signalling pathway in yeast response to acetic acid. A DNA sequence enriched in the promoter region of the Haa1p-target genes transcriptionally activated in response to acetic acid was identified and proved to be the functional binding site of this TF. A chemogenomics screening identified 400 novel determinants of resistance to acetic acid [4]. These include a number of TFs which are documented regulators of a high percentage of the genes whose expression is required for maximal tolerance to the acetic acid. They are interesting potential targets for the genetic engineering of more robust industrial yeast strains.

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Genetic variations in Il6 and LTF genes: association studies with periodontal disease

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Periodontal Disease (PD) includes inflammatory diseases induced by bacterial plaque in periodontium. PD is the most common disease of the oral cavity in domestic carnivores and 30% of the human population is affected. PD has been associated with other systemic diseases raising the interest in the investigation of factors related to its development.

This work aimed to perform a molecular analysis of interleukin 6 (IL6) and lactotransferrin (*LTF*) genes in the dog, to identify genetic variations and verify its association with PD.

The individuals (control group with 45 dogs and case group with 25 dogs) were assessed with a general clinical exam and odonto-stomatological evaluation. Genomic DNA extracted from the blood was used in the amplification of IL6 and *LTF* genes and the amplified fragments were purified and sequenced. Statistical analysis was performed with SPSS software.

We amplified two fragments for IL6 gene: 643 bp (5'-UTR-exon 2) and 630 bp (exon 5-3´-UTR). For LTF gene we amplified three fragments (630, 615 and 626 bp from exons 2, 12 and 15, respectively). We detected three genetic variations for IL6 gene (exons 2 and 5 and 3´-UTR) and eight genetic variations for LTF gene (introns 2 and 14 and exon 15). No statistically significant differences were observed between the two groups, hence no association was predicted. This is the first report on IL6 and *LTF* genes variation on dog. We pretend to analyse other candidate genes and genetic variations, in order to analyse the applicability of this model in the human PD research.

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Physical mapping of histone H3 and ribosomal DNA genes, a BAC clone, SSRs and LINE-1 in *Crassostrea gigas* by fluorescence *in situ* hybridization

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The Pacific oyster, *Crassostrea gigas*, is an economically important mollusk species cultured throughout the world. This species has a haploid complement of 10 metacentric chromosomes. Chromosomal identification is essential in genomic research. The most frequently used technique for molecular cytogenetic studies is fluorescent *in situ* hybridization which offers new opportunities for the identification of oyster chromosomes. Histone H3 gene is among the most conserved eukaryotic proteins. Major rDNA and minor (5S) rDNA genes are two families of ribosomal DNA genes in higher eukaryotes. Histone H3 and ribosomal DNA genes are repeatedly organized into clusters, which make them ideal chromosomal markers. Bacterial Artificial Chromosome (BAC) clones contain some specific regions of the genome. Simple sequence repeats (SSRs) are a class of repetitive DNA sequences widespread in eukaryotic genomes. Long interspersed nuclear elements (LINEs) are non-long terminal repeats retrotransposons and represent about 20% of human and mouse genomes (of which LINE-1 is most abundant). A preliminary physical map of *C. gigas* was produced with histone H3 gene, major rDNA (internal transcribed spacer) and minor (5S) rDNA genes, BAC clone 104B12, three different SSRs: $(GGAT)_4$, $(GT)_7$ and $(TA)_{10}$, and LINE-1. Histone H3 and 5S rDNA genes were clustered at two different *loci*. The ITS and the BAC clone 104B12 were localized at one *locus*. SSRs and LINE-1 showed dispersed hybridization extending over the length of the chromosome arms with some regions of concentration. This preliminary physical map of *C. gigas* will provide a foundation for comparative genomics in oysters.

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Interaction *Olea europaea versus Colletotrichum acutatum*

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The olive tree (*Olea europaea* L.) is a fruit species with relevant economic importance in the Mediterranean's countries agriculture. Olive anthracnose, caused by, an hemibiotrophic fungus, *Colletotrichum acutatum* damages immature, developing, and ripe olive fruits. However, the degree of tolerance to *C. acutatum* in olive cultivars is very variable, going from tolerant to susceptible. Under favourable environmental conditions, the disease can devastate entire olive orchards. To establish a compatible fungal interaction, specialised infection structures are differentiated on the cuticle, and then inside the host. The research on *O. europaea* versus *C. acutatum* aims to develop an integrated approach, using genetic, pathology, physiology, and molecular biology to understand the host-pathogen interaction. Three olive cultivars 'Galega' (susceptible), 'Cobrançosa' (moderately-tolerant) and 'Picual' (tolerant) were selected, and based on their known resistance to *C. acutatum* infection were used. Fluorescent microscopy was used to observe the pathogen infection pathway. Different infection structures were found depending on the olive cultivar, using fluorescent microscopy. Resistance aspects that can be related to the fruit cuticle (thickness, perimeter and area of epidermal cells) on tolerant and susceptible olive cultivar were analysed under light and scanning electron microscopy. Gene expression analysis during susceptible and tolerant olive cultivars attack by *C. acutatum* was accessed using cDNA-AFLP approach. Some genes have been identified and functionally characterised. However, more studies are being conducted in order to identify and characterise candidate genes that can be involved in this pathosystem.

This work was supported by the PhD grant to Sónia Gomes (SFRH/BD/25384/2005) and the Project 'POCTI/AGR/57817/2004', from the Portuguese Foundation for Science and Technology - FCT.

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Identification of descendants of an extinct bovine population from the Algarve region using molecular genetic analysis and numerical taxonomy analysis of morphological traits

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Decisions to initiate conservation programmes need to account for extant variability, diversity loss and cultural and economic aspects. Molecular markers and morphological traits were used to investigate if putative Algarvia animals could be identified for use as progenitors in a breeding programme to recover this nearly extinct breed.

46 individuals phenotypically representative of Algarvia (AG) cattle were genotyped for 27 microsatellite loci and compared with 11 Portuguese autochthonous and three imported breeds. Genetic distances and factorial correspondence analyses (FCA) were performed to investigate the relationship among Algarvia and related breeds. Assignment tests were done to identify representative individuals of the breed. Y chromosome and mtDNA analyses were used to further characterize Algarvia animals. Gene- and allelic-based conservation analyses were used to determine breed contributions to overall genetic diversity.

Standard numerical taxonomic methods were applied to a set of 183 (cows) and 170 (bulls) morphological traits, to derive average pairwise taxonomic distances among the sample of 257 cows and 76 bulls.

Genetic distance, FCA results, and UPGMA-based phenograms and a principal coordinate analysis confirmed the close relationship between Algarvia and southern Portuguese breeds.

Assignment tests classified 30 cows and three bulls were identified that could be used to reconstitute the Algarvia breed. Molecular and morphological results were concordant.

Molecular analyses complemented morphological findings to identify 33 animals that can be considered remnants of the Algarvia breed. Results of genetic diversity and conservation analyses provide objective information to establish a management program to reconstitute the Algarvia breed.

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Isolation of candidate genes conferring aluminium (Al) tolerance: A strategic approach to overcome Al toxicity in wheat

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Utilization of marginal soils by breeding tolerant plants to abiotic stresses provides an alternative strategy for agricultural expansion. Aluminium (Al) stress involves panoply of molecular mechanisms so plants may protect themselves resulting as a natural reaction. In wheat, this reaction seems to have a polygenic nature and each gene has more than one function dependent of the level of ion toxicity. Therefore, breeding for Al tolerance must include different strategies; including gene transfer from wild relatives. However, to understand the gene networks that underlie Al plant stress, it is necessary to identify and characterize the genes responsible for this stress. The main objective of this research is to identify and isolate the genes conferring Al tolerance in hexaploid wheat, mainly in previously identified extremely tolerant indigenous hexaploid wheat cultivars from Portugal. Five candidate genes were selected belonging to different gene families, namely MATE, ALMT, Zing Finger Proteins and TCA cycle genes for Al tolerance. The candidate genes have been successfully cloned and sequenced in several hexaploid wheat cultivars, including the highly Al-tolerant 'Barbela 7/72/92' line. The divergence at protein level among the different cultivars was studied for all genes. In addition, the chromosomal location of the selected genes was identified in the wheat genome using nullisomic lines. In the future, the chromosomal region identification associated with Al tolerance are going to be identified in the wheat mapping population derived from 'Barbela' and 'Anahuac' and the expression of these genes under Al stress at transcript level will be investigated.

This work was supported by the PhD grant to Ana Luisa Oliveira (SFRH/BD/45556/2008) from the Portuguese Foundation for Science and Technology (FCT).

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Candidate genes for cat mammary tumor: a survey from comparative genomic hybridization analysis

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An interesting opportunity for comparative oncology has been recognized in spontaneously occurring tumors in domestic animals. Particularly, cat mammary tumors (CMTs) are considered fine models for their human counterpart. However, data regarding CMTs cytogenetic characterization is very scarce, most specially at the molecular level.

Genetic aberrations, such as gene amplifications, deletions, and loss of heterozygosity are hallmarks of cancer and are thought to be major contributors for the neoplastic process. The advent of Comparative Genomic Hybridization (CGH) has opened a reliable way for detection of genomic imbalances in each tumor through a single analysis. Its utility is based on the concept that chromosome regions with increased sequence copy-number reveal sites that may contain dominantly acting oncogenes, whereas regions with decreased sequence copy-number may harbor putative suppressor genes.

To date, up to 5-10% of all human breast cancers are caused by germ-line mutations in well-identified breast cancer susceptibility genes. These genes can be roughly divided into “high risk” (BRCA1, BRCA2, PTEN, TP53, LKB1/STK11 and CDH1) and “low to moderate risk” (CHEK2, TGFβ1, CASP8 and ATM).

In the present work, we present the analysis of five CMT cases by CGH, allowing the detection of most significant genomic imbalances (gains and losses). Several Comparative Maps (i.e. chromosome paint and radiation hybrid maps) were used to infer the physical location of the “high-risk” and “low to moderate risk” of the homologous cat breast cancer genes. This *in silico* analysis uncovered an interesting correlation between the genomic imbalances detected and cancer critical genes co-localized.

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Different organization patterns of a satellite DNA sequence in closely related species, *Phodopus sungorus and Peromyscus eremicus* (Rodentia, Cricetidae)

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An important feature emerging from the work on several experimental organisms is that satellite DNAs and other repetitive sequences can act as ‘‘active agents’’ for chromosomal evolution in mammals, implicated in reorganizational processes [1]. Moreover, different authors suggest that satellite DNA sequences can even promote chromosomal rearrangements, due to their rapid evolution by means of intragenomic movements among different chromosomal fields [2].

Here we report the isolation of a *Phodopus sungorus* satellite DNA sequence, obtained from a repetitive DNA library constructed after a series of experimental steps: restriction of *P. sungorus* total genomic DNA, cloning of the restriction products and colony-lift hybridization using the restriction products as probes.

Physical mapping by fluorescent *in situ* hybridization located this sequence exclusively on centromeric positions of *P. sungorus* chromosomes. Interestingly, when the physical location of this sequence was investigated on chromosomes of other Cricetidae species, *Peromyscus eremicus*, a scattered pattern of distribution was observed throughout the karyotype. The presence of this repetitive sequence in *Phodopus sungorus* and *Peromyscus eremicus* implies its existence in a common ancestor. The existence of orthologous repetitive DNA sequences displaying different chromosomal locations in the two Cricetidae genomes suggests the occurrence of intragenomic movements of this repetitive sequence, resulting in an extensive process of karyotype restructuring during Cricetidae genome evolution.

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An orthologous satellite DNA sequence between Muridae and Cricetidae

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Satellite DNA (satDNA) sequences seem to be located at genomic regions prone to chromosome rearrangements, and it is believed that, somehow, their dynamic nature promotes chromosomal evolution [1].

SatDNA sequences are a common feature of higher eukaryotes and in a given genome several unrelated satDNA families may coexist. Each of these families is usually confined to one or more groups of phylogenetically related species, but some of them can be found in phylogenetically distant species, suggesting evolutionary conservation [1].

The highly repetitive DNA component – the satellite I (satI) family, from the laboratory rat (*Rattus norvegicus* (RNO) (Rodentia, Muridae)), is composed of tandemly repeated 370 bp monomers [2]. In the present study we succeeded in isolating this satDNA family (or variant) from a RNO phylogenetically distant species - *Cricetus cricetus* (CCR) (Rodentia, Cricetidae). Physical mapping, with species-specific satI probe, showed a different genome distribution in the two species: while in RNO chromosomes this satDNA sequence is localized at the peri(centromeric) regions of some RNO chromosomes, in CCR genome this satDNA sequence variant displays an interstitial distribution.

SatI sequence seems to change slowly with the course of evolution. We suggest a (peri)centromeric location of this satellite DNA family in the Muroidea ancestral karyotype and, in the course of CCR genomic evolution, the evolutionary rearrangements reshuffled its genomic location.

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Cytogenetic characterization of the dwarf oyster *Ostrea stentina* (Mollusca: Bivalvia) and comparative karyological analysis within Ostreinae

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Regardless of the high economic value and large geographic distribution of oysters, the current knowledge of oyster taxonomic relationships and systematics is still limited, particularly for flat oysters. In this study, the molecular cytogenetic characterization of mitotic chromosomes of the Provence flat oyster or dwarf oyster *Ostrea stentina* was performed through Giemsa staining, chromosome measurements, *in situ* restriction endonuclease banding, C-banding, DAPI staining and fluorescence in situ hybridization (FISH) with major ribosomal RNA genes (rDNA) and telomeric sequence (TTAGGG)n. The karyotype (2n=20) consisted of six metacentric (1, 3, 4, 6, 8 and 10) and four submetacentric (2, 5, 7 and 9) chromosome pairs. Chromosome treatment with HaeIII produced specific banding patterns for all chromosomal pairs, confirming the efficiency of this restriction enzyme for chromosome banding in oysters. Results on C-banding revealed the presence of heterochromatin in the telomeric regions of the short arms of a large metacentric and a submetacentric chromosome pairs. In situ hybridization with telomeric sequence revealed bright hybridization signals in the telomeres of all chromosomes. The location of the major ribosomal rDNA displayed the presence of two signals, in the telomeric regions of the short arms of a largest metacentric chromosome and in a submetacentric chromosome. The cytogenetic data obtained was used to perform a comparative karyological analysis within the subfamily Ostreinae. It is also important to highlight that this type of work can provide new insights on major genomic changes at chromosome level within the flat oysters.

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Poster Communications

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Screening of iron-chelating compounds

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The rational use of miniaturized devices is contributing to improve bioprocess development. Along, with this, the screening of natural compounds for application in biotechnology has been anchored on land-based samples. However, new exploratory technologies have broadened the access to sampling sites of natural compounds for application in biotechnology that were inaccessible until recently, among which the marine environment. The full extent of marine biodiversity is far from being known and the number of potential producers of commercially interesting bioactive compounds is vast.

Among these are siderophores which are high-affinity iron-chelating compounds with applications ranging from the medical field to the environmental sector. Due to their ability to bind heavy metals, they can also be eligible for remediation of metal contaminated sites or industrial waste.

This work aims to obtain a collection of environmental bacteria with the ability of producing siderophores and to develop a process intensification platform, anchored in microtiter plates, to isolate the siderophore producing strains. Strains were collected along the Portuguese continental shelf and estuaries.

The platform was able to isolate strains in less than a week. Furthermore, different separation techniques were further developed for the extraction and purification of the different siderophores from the production broth, which will allow the consequent analysis of product purity and its characterization.

Six of the isolated strains presented a 10-fold increase in production yield when compared with data obtained for an over-producing reference strain. The siderophore production process is being developed to increase the overall siderophore yield to industrially interesting values.

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Phenyl-boronate membrane affinity chromatography as an approach to intensify plasmid DNA purification

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The current state of the art of pDNA as a third generation biopharmaceutical has fostered the development of a myriad of preparative chromatographic techniques [1]. Among them phenyl-boronate ligands immobilized on porous glass (74 μm ≤ f ≤125 μm) have been successfully explored to purify pDNA [2] . Phenyl boronate groups interact with molecules containing two vicinal hydroxyl groups in *cis* configuration, via the formation of two covalent bonds in a five-membered ring complex. This property was used to explore one of the few chemical differences between RNA and DNA, i.e. the presence of vicinal 2', 3' *cis*-diol at the 3' end of the RNA molecule [3]. Since the 3'-hydroxyl is absent in DNA, plasmids will not esterify with boronate matrices, making it possible to separate pDNA from RNA. A direct application of alkaline lysates on this type of beaded matrices allows the removal of most of the RNA (the most abundant impurity) and endotoxins by affinity interactions. Moreover, some proteins and genomic DNA were also removed, although not specifically [2].

This work main focus is the coupling of phenyl-boronate ligands in cellulosic membrane adsorbers and their characterization on the chromatographic ability to retain RNA impurities and purify pDNA in flowthrough mode, in accordance to what was previously performed with the phenyl boronate glass beads. The fractions of the peaks obtained in the chromatographic experiments were collected and further analysed by agarose gel electrophoresis, HPLC, RT PCR and LAL assay.

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Potential Application of PEG600-Phosphate Aqueous Two-Phase Systems in the purification of Leguminosae family lectins

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Lectins hold great promise not only as reagents for diagnostics and drug discovery but also as biopharmaceutical products. In fact, new research directions in the last years have led to major developments in the uses of plant lectins as therapeutic agents against numerous diseases in an ageing society. All these new trends are placing a tremendous emphasis on the development of new approaches for faster lectins development, selection and optimization, including alternatives methods of purification.

In general, the purification of lectins from crude extracts has been made through several chromatographic steps, comprising an affinity chromatography step, as the first step, which can be relatively expensive and leads to low recovery yields (less than 50%). An effective, rapid and cheap methodology, for the purification of the lectin from *Canavalia brasiliensis* (ConBr) seeds, based on aqueous two-phase systems (ATPS) using a design of experiments (DoE) for process optimization was developed. A polyethylene glycol (PEG) 600-phosphate aqueous two-phase systems (ATPS) containing NaCl was found to selectively extract ConBr into the PEG phase. The best conditions of purification were achieved using an ATPS composed by 16.5% (w/w) PEG 600, 15.0% (w/w) phosphaste pH 7.5, 4.5% (w/w) NaCl. The maximum percentage yield of protein extracted was about 100% with a final purity of 73.04%. This technique was further expanded to the recovery other lecins from different sources [1,2]

Acknowledgment: National Counsel of Technological and Scientific Development (CNPq)

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Purification of human antibodies using gum arabic coated magnetic particles

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Gum arabic coated magnetic particles derivatized with different ligands were assessed for their binding capacity towards human IgG. Gum Arabic is a biocompatible natural composite polysaccharide that provides functional groups (e.g. carboxylic acids, amines) for further surface modification of the magnetic particles [1]. The magnetic adsorbents were covalently coupled with both affinity and ionic exchange ligands. The affinity ligand comprised the phenyl boronic acid that specifically recognizes *cis*-diol-containing molecules, such as glycoproteins. Antibodies are a part of this group of proteins as they bear oligosaccharides in both the Fv and Fc regions. In the Fc region, fucose, galactose and mannose, all containing 1,2-cis-diol groups, can be typically found. Another approach was to use nitrilotriacetic acid (NTA) as a cationic exchanger due to the high isoelectric point of the IgG used.

The binding selectivity of the magnetic adsorbents was evaluated in a model protein mixture containing human IgG, albumin and insulin. In cell culture media proteins such as insulin and transferrin are typically found to support cell growth, namely in glucose metabolism and iron delivery, respectively. Ultimately, the feasibility of using the aforementioned magnetic adsorbents in the direct purification of IgG from a CHO and hybridoma cell culture supernatant was evaluated.

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Desulfurization of Crude Oil Compounds by *Rhodococcus erythropolis* Cells in Biphasic Media

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Refineries are now facing the challenge of simultaneously keeping up with the increasingly global demand for energy and the stringent limitations on SOx emissions. Biodesulfurization (BDS) is a process that uses microorganisms, such as *Rhodococcus erythropolis*, as the catalyst for fuel desulfurization. The feasibility of using two different strains of *R. erythropolis* for the desulfurization of crude was investigated using dibenzothiophene (DBT) as model compound.

The effect of several solvents such as hexane, heptane, octane, nonane, decane, dodecane and hexadecane upon whole cells *R. erythropolis* was investigated to determine which could be used as DBT reservoir in organic:aqueous systems. The toxicity of both solvents and DBT to the cells was determined by analysis of cell viability using fluorescence microscopy techniques. The results showed that hexane is far more toxic than hexadecane for *R. erythropolis* IGTS8 cells. In the presence of hexadecane, cells produced surfactants and showed to be resistant up to a concentration of 160mM of DBT. In the biphasic systems, the capability of the bacteria to desulfurization could be evaluated as much higher DBT concentrations were possible when compared to aqueous systems. In the latter, *R. erythropolis* cells were able to metabolize 0.1mM of DBT in 2 hours. With biphasic systems (Water/Hexadecane), which better mimic the real system, it was possible to dissolve and rapidly metabolize a higher quantity of DBT without losing the cell's viability. In this system, the cells were able to fully metabolize 14.4mM of DBT in 24hours.

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Mesoporosity Generation in Nu-10 by Desilication

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Mesoporous TON zeolite samples, preserving the crystallinity, were obtained by treating a purely microporous sample, in alkaline medium with NaOH [1], at 80ºC, with different concentrations and treatment times. The samples were characterized by nitrogen adsorption. Treatment with alkaline solutions at 0.05M and 0.2M induced an enhancement of the external surface and a slight increase of the, probably intercrystallite, mesoporosity. Under more severe conditions, 0.4M and 0.6M, the samples presented hysteresis loop, indicating the presence of internal mesopores. Toluene adsorption experiments were also performed. The majority of the samples presented a higher toluene adsorption capacity, comparing to parent zeolite. Contrarily, the samples treated at 0.6M during 45min and 60min revealed lower toluene retention, which could be due to some extra-framework species that remain in the internal porosity. This is in agreement with the pronounced increase of the Lewis acid sites amounts in these samples.

Catalytic tests were accomplished with desilicated samples presenting higher internal mesoporosity than the parent zeolite, in the shape selectivity sensitive model transformation of xylene isomerization. Comparing the results obtained for the different samples, a slight decrease in the initial p-xylene isomerisation activity, at 400ºC, taken at 2 min time-on-stream, was noticed, being probably related to some decrease in the protonic sites concentration. Nevertheless, the activity per acid site is, in some desilicated samples, increased, relative to the parent zeolite, suggesting a probable easier reactant accessibility. Furthermore, the para/meta-xylene molar ratio decreased and, so, the zeolite shape selectivity. On other hand, there is an increase of the trimethylbenzene yield, which could be also ascribed to higher internal space available and to an easier diffusion inside the HNU-10 porosity, allowing the appearance of products resulting from the bimolecular xylene dismutation.

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Characterization of the Wave bioreactor: Residence time distribution

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The high dose requirements of biopharmaceutics have led to the development of mammalian cell culture technologies to increase biomanufacturing capacity. Among them, disposable bioreactors are attracting attention, particularly the Wave bioreactor. This system induces an undulation movement to the culture, ensuring good mixing and oxygen transfer without shear damage, and requires no cleaning/sterilization, providing simpler operation and no cross-contamination. However, this new reactor still needs further characterization. In this sense, the residence time distribution (RTD) was evaluated, allowing the characterization of the mixing/flow and the comparison with ideal models and a commercial stirred tank reactor (STR). RTD was determined using methylene blue with a pulse input methodology, at three mammalian culture flow rates: low (L: $3.3x10^{-5}$ m^3/h), intermediate (I: $7.9x10^{-5}$ m^3/h), and high (H: $1.25x10^{-4}$ m^3/h). Samples were taken and absorbance read at 660 nm. Results show that Wave behaviour approximates the ideal and experimental STR at flow L, but deviates from ideal models at flows I and H. The comparison of average residence time (tr) with time of passage (τ) provides a possible explanation for this non-ideality. For STR at all flows and Wave at flow H, tr was lower than τ, indicating dead zones inside the reactor. For Wave at flows L and I, tr was higher than τ, indicating short-circuiting.

In conclusion, the choice of flow rate will strongly influence the behaviour of the Wave bioreactor. The use of a low flow seems to be a choice that provides behaviour closer to the ideal continuous STR model.

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Growth enhancement of benthic diatoms for industrial applications

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Diatoms are photosynthetic unicellular algae found in aquatic environments.[1] They have many (potential) industrial applications due to their position at the basis of the aquatic food chain, peculiar fatty acid composition and intricately ornamented siliceous cell wall.[2] This study aimed at the growth enhancement of benthic diatoms in tree-dimensional batch cultures using filamentous substrates.

Achnanthes intermedia and *Eunotia bilunaris* were grown, respectively, in F/2 and WC medium conditioned with filamentous cellulose DIACELL® 1000 and glass fibers. The effect of these substrates was evaluated by fluorimetry, dry and carbon weight analysis (this latter only for glass fibers). Two different experiments were performed: (1) growth rate and final biomass increase (stationary growth phase) and (2) biomass increase at the end of exponential growth phase. It was found that both substrates lead to a significant increase in growth rate and in final biomass. The biomass increase was higher in cellulose conditioned cultures especially in case of *Eunotia bilunaris*.

This study showed that filamentous substrates increase the carrying capacity of cultures by offering a suspended attachment surface. Moreover, an increase of biomass in suspension comparing to the control was observed, leading to easier harvesting. This method presents an opportunity of scaling-up diatoms cultivation, increasing even further the biomass production.

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Biotechnological Versatility of the riboflavin producer *Ashbya gossypii*

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The ascomycete *Ashbya gossypii* is a natural producer of riboflavin closely related to *Saccharomyces cerevisiae*. Most of the studies related to this organism are either focused on riboflavin production or in the study of polarized hyphal growth on the molecular level. We further studied *A. gossypii* physiology by evaluating the similarities and differences of three related *A. gossypii* strains, ATCC10895, MUCL29459, IMI31268 and the more distant CBS109.26 [1]. Although A. *gossypii* ATCC10895 is often described as wild type, it differed from the parent strain, IMI31268, in specific and in colony radial growth rate on different carbon or nitrogen sources. In addition, although MUCL29450 was deposited as ATCC10895, it has clearly diverged from it, showing significant differences from the parental strain IMI31268 than ATCC10895. All strains tested had greater sensitivity to low pH than most filamentous fungi.

Apart from the physiological characterization of *A. gossypii*, the potential to produce valuable compounds, besides riboflavin, was explored. Cloning and expression of the heterologous proteins, endoglucanase I (EGI) and cellobiohydrolase I (CBHI) from *Trichoderma reesei* was achieved in *A. gossypii* ATCC10895*,* allowing to study the ability of this fungus to produce recombinant proteins [2]. Both proteins were secreted into the culture medium. Nonetheless, more EGI was secreted than CBHI, or more active protein was produced. Partial characterization of CBHI and EGI expressed in *A. gossypii* revealed overglycosylation when compared to the native *T. reesei* proteins, but less extensive than on cellulases expressed in *S. cerevisiae*. Therefore, the expression of recombinant cellulases in *A. gossypii* provides opportunity for future development of *A. gossypii* as a promising heterologous protein production host.

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A novel *Escherichia coli* fusion system for production of recombinant immunogenic proteins

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Recombinant protein production is a useful technology for therapeutic and diagnostic applications, namely for polyclonal antibody production. Antibodies are usually raised against a specific protein by immunization of animals with the purified protein. The bacterium *Escherichia coli* has been widely used for the bio-production of proteins, but it still presents some drawbacks: many proteins of biomedical interest are difficult to express properly in this host system, resulting in insoluble protein aggregates. Gene fusion technology has been applied to optimize recombinant protein production in *E. coli*. Fusion partners have also been used to potentially increase protein immunogenicity.

A novel fusion system (Fh8 and H tags) was recently discovered and demonstrated to improve antigen production in *E. coli* [1], presenting also attractive features for the development of antibodies. In this work we explored the immunopotentiating properties of H tag using a 12 kDa surface adhesion protein of *Cryptosporidium parvum* (CP12) [2] as model antigen. Production yields of Fh8CP12, HCP12 and CP12 recombinant proteins were evaluated and polyclonal antibodies were raised against both CP12 and HCP12 antigens. Results obtained here demonstrated that the fusion of both Fh8 and H tags to CP12 improved its expression in comparison with non-fused CP12 protein, and that H tag efficiently increased CP12 specific immunogenicity without being removed from the fusion antigen and without co-administration of adjuvants, resulting in a more effective and earlier immune response.

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Light regime characterization in a photobioreactor for microalgae production using optical fibre technology

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The slow development of microalgal biotechnology is due to the failure in the design of large-scale photobioreactors (PBR) where light energy is efficiently utilized. In this work, both the quality and the amount of light reaching a given point of the PBR were determined and correlated with cell density, light path length and PBR geometry. This was made for two different geometries of the downcomer of an airlift PBR using optical fiber technology that allows obtaining information about quantitative and qualitative aspects of light patterns. This is important since the ability of microalgae to use the energy of photons is different, depending on the wavelength of the radiation. The results show that the circular geometry allows a more efficient light penetration, especially in the locations with a higher radial coordinate (*r*) when compared to the plane geometry; these observations were confirmed by the occurrence of a higher fraction of illuminated volume of the PBR for this geometry. An equation is proposed to correlate the relative light intensity (RLI) with the penetration distance (*Pd*), for both geometries and different microalgae cell concentrations. It was shown that the attenuation of light intensity is dependent on its wavelength, cell concentration, geometry of PBR and the penetration distance of light.

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Purification of fructo-oligosaccharides

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Fructo-oligosaccharides (FOS) have gained large commercial interest due to its beneficial properties in the human health as prebiotics. When a fermentative process is used to produce FOS, the removal of salts and low molecular weight sugars from the fermentation broth is required. In this work some of the techniques used to separate FOS from low molecular weight sugars were evaluated and compared.

The application of size exclusion chromatography with Bio-gel P2 allowed the complete fractionation of all sugars enabling the chemical, clinical and nutritional characterization of each single FOS; however, very low recovery yields were obtained. On the other hand, an efficient demineralization of the broth and a recovery of 80% of FOS with 89% of purity were obtained when the separation was conducted in an activated carbon column, operated in batch mode, using ethanol gradients.

Although the mentioned processes proved to be efficient and simple, both are laborious and time consuming if implementation in an industrial scale is envisaged. Therefore, other techniques ought to be explored. Simulated Moving Bed (SMB) appears to be an alternative due to its great productivity and continuous mode of operation. Accordingly, experiments were conducted in order to choose an adequate and efficient resin. Based on the experimental determination of the adsorption isotherms of several resins, Dowex Monosphere 99K/320 was selected. This resin was tested in a SMB unit and, experimental and *in silico* data were compared. The obtained results suggest that SMB is a useful technology for purifying FOS from fermentation mixtures at an industrial scale.

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Optimization of Bioethanol Production by a Flocculating *Saccharomyces* cerevisiae using Simultaneous Saccharification and Fermentation Technology

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Bioethanol production from lignocellulose sources such as wheat straw (WS) requires enzymatic hydrolysis of cellulose to release sugars (saccharification) that can subsequently be fermented by yeast to ethanol. One way to perform this reaction at high dry matter condition is by a simultaneous saccharification and fermentation (SSF) procedure [1]. On other hand, in order to increase the fermentable sugars concentration it is necessary to improve the availability of cellulose in the substrate by removing non-cellulosic materials under different kind of pretreatment. For that reason the aim of this study was to use WS biomass as lignocellulosic raw material fractionated by a hydrothermal pretreatment for being used in a batch SSF process for ethanol production, evaluating the effect of substrate, temperature and enzyme loading. Substrate for SSF (WS), was treated under autohydrolysis process (180 ºC/ 20 min) [2]. A full factorial design 2^3 was used for the optimization of the pretreated WS to improve SSF ethanol production. The highest ethanol yield was 78.4%, respect to the theoretical conversion yield at 45 ºC, 2.5% of substrate and 17.35 FPU/mL of cellulase. Ethanol can be successfully produced from wheat straw by SSF.

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Enhanced growth of *Pichia pastoris* under increased air pressure on different carbon sources

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Pichia pastoris has many biotechnological applications. Two aspects of the species have contributed to its utility: (1) fermentation techniques were developed for maintaining extremely high cell densities in excess of 100 g/L dry weight, and (2) because *P. pastoris* assimilates methanol, the expression system is linked with alcohol oxidase, which is abundantly produced in the presence of methanol.

The high oxygen demand of methanol metabolism and cultivation at very high-cell-density makes oxygen supply a major parameter in *Pichia pastoris* cultivation. Previous work demonstrated that hyperbaric air could be successfully applied to yeast cultivation, as a way of improving the oxygen transfer rate (OTR) to aerobic cultures [1].

In the present work, we investigate whether increasing air pressures may lead to increasing biomass yields of *P. pastoris*, growing with four carbon sources, without giving rise to unbalance oxidative stress.

Pichia pastoris strain was grown in glucose, pure glycerol, crude glycerol from biodiesel industry and methanol media under total air pressure from 1 bar to 5 bar. In all the experiments, the cultures reached maximum cell density at 5 bar of total air pressure. A 4-fold increase on specific growth rate was obtained at 5 bar on glycerol and crude glycerol compared to the value at atmospheric pressure. Biomass yield was also enhanced by air pressure rise, for all carbon sources. With 5 bar air pressure biomass yield (g cells/g carbon) was 0.97 and 1.86 whereas at 1 bar was 0.67 and 0.77, respectively in methanol and glycerol media.

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Salt Effect on the Aqueous Two-Phase System PEG 8000 - Sodium Sulfate: Physico-Chemical Characterization of the Systems

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In recent studies there is one issue that seems to be one of the most interesting in regard to partitioning of biomolecules, from both theoretical and practical viewpoints - the effects of salt additives on partition [1]. Zaslavsky [2] studied the effect of several salts on polymer-polymer ATPSs and stressed on the role of "water structure" as an important factor controlling two-phase formation. In polymer-salt ATPSs, this issue has never been examined systematically. The main goal of this work is to study the effects of different salt additives (NaCl or KCl), with concentration up to 1.0 M, on PEG 8000 - Na_2SO_4 ATPS, containing 0.01 M of sodium phosphate buffer, pH 7.4, at 296.15 K. Phase diagrams determined by the cloud point method, including tie-lines assigned from mass phase ratios according to the lever arm rule, are presented. The results indicate that the salting-out ability of the cations follows the Hofmeister series ($Na^+ > K^+$) and can be related to the ions Gibbs free energy of hydration (ΔG_{hyd}).

The Gibbs free energy of transfer of a methylene group between the coexisting phases, $\Delta G(CH_2)$, was been used to characterize the difference between the hydrophobic character of the equilibrium phases of those particular ATPSs. The $\Delta G(CH_2)$ *was* determined by partitioning of a homologous series of five sodium salts of dinitrophenylated - amino acids with aliphatic side chains in different tie-lines of each biphasic system. The results show that, within each system, there is a linear relationship between the $\Delta G(CH_2)$ and the tie-line length.

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Physicochemical characterization and extraction of bioactive compound from *Larrea tridentata* leaves

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Larrea tridentata plant is originally from arid regions of Northern Mexico and South-western of United States and is commonly known as creosote bush or chaparral. This plant is a notable source of a bioactive compound called nordihydroguaiaretic acid (NDGA) with important biological activities of great interest in the health area [1]. The purpose of the present study was to perform a physicochemical characterization of *Larrea tridentata* leaves, and to evaluate the effect of different organic solvents on NDGA extraction and antioxidant capacity of the extracts.

A high content of total lignin (35.96%) was found in *Larrea tridentata* leaves compared with other fractions, such as cellulose and hemicelluloses (10.09 and 13.10%, respectively). *Larrea tridentata* leaves contained 13.01% protein, 2.62% acetyl groups and 7.91% ash. NDGA extraction varied considerably according to the used solvent. Heating played an important role in NDGA recovery when using methanol; but did not influence the extraction with ethanol or acetone. The highest NDGA content (46.96 ± 3.39 mg/g DW plant) was recovered using 90% methanol. However, the highest total phenolic content (487.13 ± 27.68 mg GAE/g DW plant) was obtained using 90% acetone. All the extracts showed antioxidant capacity with similar results for DPPH radical scavenging activity. Different behavior was observed for FRAP results where extracts obtained using 50% and 90% methanol had significantly higher (p<0.05) values (2.58 ± 0.10 and 2.77 ± 0.19 mM FE(II)/ g DW plant, respectively) than the remaining extracts. These high antioxidant activity values for FRAP assay might be explained by the high TPC and NDGA concentrations in both extracts.

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Development of a continuous perfusion bi-directional bioreactor for large sized constructs

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A new bioreactor named Continuous Perfusion Bi-directional Bioreactor (CPBB) was developed to optimize flow directions in the interior, as well as on the exterior of large sized constructs. The flow directions in the bioreactor are being regulated by a peristaltic pump (positive force) and with a vacuum pump (negative force). To determine the feasibility of this bioreactor, constructs composed of 14 mm diameter SPCL (a blend of starch and polycaprolactone) fiber meshes were seeded with goat marrow stromal cells (GMBCs). After 14 days of culture in the bioreactor at a flow rate of 1 ml/min, constructs were analyzed on morphology (SEM), proliferation (DNA assay) and differentiation (ALP activity) of the cells. The results of the SEM analysis showed that cells adhered to the surface of the SPCL fiber meshes and that the cells were well-spread. The DNA amount of the cells cultured in the bioreactor showed significant lower values compared to static cultures. However, the ALP activity indicated better values compared to the static cultures. The lower values of the DNA amount of the constructs in the bioreactor could be explained by shear forces in the constructs, thereby hampering cell proliferation but stimulating cell differentiation.

Protein adsorption and cellular interactions on nano-structured polyelectrolyte multilayer films

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Surface modification constitutes a novel approach to improve biomaterials integration in biologically complex systems. The incorporation of bioactive molecules opens a new route for coating functionalization that can be applied in implants, prostheses, tissue engineering and, in the future, have clinical applications in cancer therapy [1]. Under this model study, the adsorption of human serum albumin (HSA) onto chitosan-alginate multilayered assemblies was assessed *in-situ* by quartz crystal microbalance with dissipation monitoring (QCM-D). It was found that the behaviour of HSA on biomaterials surface can be tuneable by adjusting some physicochemical parameters of the underlying polyelectrolyte system such as pH, layer number, crosslinker and polymer terminal layer. Our results confirmed the key role of electrostatic interactions during HSA adsorption, since surfaces with opposite sign charge were more effective to promote protein adhesion. Additionally, our data indicated that crosslink enhanced the stability of chitosan-alginate films and, simultaneously, enabled the adsorption of HSA under physiological conditions (pH 7.4, NaCl 0.15 M). Moreover, crosslinking the top layer of the assemblies did not seem to modify sufficiently the film mechanical properties in order to improve cell adhesion and proliferation. Our results suggested that the biological potential of biopolymers and the mild conditions of the LbL technique turn these natural nano-assemblies into a suitable choice to be used as pH-sensitive coatings.

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Thermal resistance of cork components

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Cork is the outer bark of the Oak tree *Quercus suber* L and has been widely applied in the construction field; automobile and aerospace industries. The suitability of cork for this wide range of applications is supported by its properties, namely: hydrophobicity, elasticity, very high recovery, near-zero Poisson coefficient and thermal resistance [1, 2].

The main chemical components of cork are: suberin, lignin, polysaccharides, waxes and polyphenols. From those components, suberin presents the greatest portion in cork (up to 50%), followed by lignin (up to 29%); polysaccharides (up to 25%) and the extractives (up to 24%) [1].

The thermal behaviour of cork is one of its most interesting properties. It is considered as a thermal insulator, being used as a protective blocker of thermal fluxes. Taking this into consideration, it is relevant to analyze which are the components that are responsible for this property and to evaluate their thermal stability.

We selectively extracted the compounds present in cork and evaluated their thermal resistance using thermogravimetric analysis (TGA). Additionally, the extracted cork was also analysed as a function of the remaining components.

It was possible to identify the thermal degradation of suberin, lignin, polysaccharides and some of the compounds present in the extractives fraction. In particular, the degradation of suberin occurs at ~430ºC, before lignin degradation that only occurs at ~450ºC. Overall lignin is the cork component more resistant to thermal degradation followed by suberin. The polysaccharides and extractives degrade at a wider temperature interval that range from 200ºC to 420ºC.

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Buildup of biomimetic and multiple stimuli responsive thin coatings of polysaccharides and recombinant biopolymers

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The modification of surfaces has been used in cutting-edge applications to produce smart or stimuli-responsive and patterned surfaces that may be used to develop several biomedical technologies [1]. In this work, smart thin coatings using chitosan and recombinant elastin-like polymers (ELP) containing the cell attachment sequence RGD were fabricated in a sequential fashion through layer-by-layer adsorption. The adsorption of each layer was followed *in situ* using a quartz-crystal microbalance with dissipation monitoring and showed that both polymers can be successfully combined to conceive multilayer coatings.

Because ELPs are a class of biomaterials exhibiting smart properties in solution [2], the smart properties of the coatings were tested for their wettability by contact angle measurements (CAs) as a function of several stimuli, namely temperature, pH and ionic strength. Wettability transitions were observed from a moderate hydrophobic surface (CAs approximately from 62º to 71º) to an extremely wettable one (CA considered to be 0º) as the temperature, pH and ionic strength were raised above 50ºC, 11, and 1.25M, respectively. Atomic force microscopy was performed to assess the coating topography, showing the formation of larger and compact micelle-like structures upon the aggregation of ELPs at the surface, increasing its water affinity.

Cell adhesion tests were conducted using a SaOS-2 cell line. Enhanced cell adhesion was observed in the coatings, as compared to a coating with a chitosan-ending film and a scrambled RDG biopolymer. The developed films have great potential as biomimetic coatings of biomaterials for different tissue engineering applications, controlled delivery systems and tunable cell adhesion.

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Studies of mammalian cell adhesion with bulk acoustic wave sensors

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Cell culture has become an increasingly important research platform in several scientific areas. For the *in vitro* survivor and proliferation of a cell culture, anchorage-dependent cells must adhere to a tissue culture surface and spread. The quartz crystal microbalance (QCM) is being used by us as a technique to study, in real-time and non-invasively, the ability of different mammalian cell lines (HEK 293T, HeLa and COS-7 cells) to attach and spread on the bare gold sensor's surface, in the presence and in the absence of serum proteins. Initially, serum-free or serum-supplemented medium was injected in the QCM's chamber and frequency and resistance signals were allowed to stabilize. Cells were injected and processes of cell adhesion/spreading were followed over several days, through the monitoring of the resonance frequency and resistance. In serum free-medium, cell injection induced a decrease in frequency for all cell types, meaning that cells are adsorbing at the sensor's surface. Viscoelastic changes cell related are responsible for the resistance increase. For COS-7 cells, a considerable difference is noticed for the initial resistance values, meaning that important phenomena are occurring at this stage. Experiments with HEK 293T and COS-7 cells in serum supplemented medium demonstrates that those cells behave differently in the presence and in the absence of serum, having smaller frequency and resistance shifts in the later case. Behaviour differences are even more obvious for COS-7 cells, where an increase of frequency can be observed prior to its decrease. The presence of cells at sensor's surface was confirmed by fluorescence microscopy.

Using the Quartz Crystal Microbalance to investigate DNA conformation

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DNA-based sensors represent a very active research field since gene analysis is crucial for the diagnosis of hereditary and infectious diseases, the understanding and manipulation of genome packaging and gene regulation.

The quantitative determination of size, shape and conformation of surface bounded biomolecules can be further achieved from the acoustic wave propagation coupled with hydrodynamics equations solved at the interface. Acoustic techniques, unlike other techniques, present the advantage of being sensitive to intrinsic mechanical properties of adsorbed layers [1]. While several models and solutions exist for different boundary conditions, a model based on classical polymer solution theory was recently proposed to infer conformational alterations of surface bounded DNA. The model is based on the fact that biomolecules such as DNA behave as discrete molecules, and can, thus, be modeled using classical solution viscosity theory. The combination of classical solution viscosity theory with acoustic measurements was shown to be very effective in quantitatively distinguishing between DNA of different shapes as well as in the detection and prediction of DNA conformational changes occurring upon protein binding.

Considering this new model, we are using the quartz crystal microbalance to analyze the conformation of DNA. We rely on impedance analysis of the sensor to assess the variations of the model parameters and correlate them to the physical phenomena and chemical properties of the adsorbed media at the surface. Insights regarding the rigidity of the films are thus obtained which enable the assessment of immobilized DNA bending owing to protein-DNA binding.

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Amorphous silicon photodiode as a platform for single-event detection

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Photodetectors based on hydrogenated amorphous silicon (*a*-Si:H) show high quantum efficiency in the visible, low dark current, low-temperature (below 250 ºC) processing technology that allows the use of substrates such as glass and polymers. These attributes make them attractive candidates for application in integrated biosensor arrays. Furthermore the miniaturization to the micro scale results in a biochip that allows sensitive, rapid and real-time measurements, also leading to a flexibility of portability. In the present work, this integrated platform is used to optically detect biomolecular interactions in which a species is labeled with semiconductor quantum dots (QDs). An amorphous silicon-carbon (a-SiC:H) filter, integrated in the chip, cuts the excitation light while allowing the transmission of the fluorescence light emitted by CdSe/ZnS core-shell QDs at ~600 nm, which produces a photoresponse on the a-Si:H layer. The detection limit of the present device is of the order of 0.01 pmol for the QD600 in solution (corresponding to a concentration of 10^{-9} M). Common biomolecular interactions such as streptavidin with biotin were successfully detected. We are developing a more specific application of these detectors which is the detection of the HIV-1 Vif (virion infectivity factor). Anti-HIV-1 Vif single chain fragment antibodies (scFv-4BL) were developed and immobilized on the surface of the detector chips. HIV-1 Vif is detected by the fluorescent signal after being conjugated to QDs.

A membrane protein case study - the (in) stability of purified CYP3A4

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Membrane proteins have long been recognized as major drug targets. The studies of their structure and function have been scarce, though, as they become highly unstable in the required purity for structural and functional studies.

Cytochromes P450 are membrane proteins of particular interest as these enzymes are involved in the metabolism of a wide array of therapeutic drugs and intoxicants, of which CYP3A4 is the most abundant of all human P450s.

In this communication we use CYP3A4 as a case study to illustrate the difficulties of the production and purification of membrane proteins. We show that purified CYP3A4 is highly unstable, decaying rapidly to a inactive conformation of the enzyme using Fluorescence, Fe2+-CO minus Fe2+ spectra, and activity assays towards testosterone. Results show final protein preparations to be very unstable, having a Tm of approximately 300K (27ºC), in the presence of 20% glycerol.This inactivation of soluble preparations may hamper any scientific study requiring the purified enzyme.

Acoustic impedance analysis to study biomolecular recognition

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Acoustic wave sensors have been shown to be highly effective functional devices to monitor biomolecular binding in real time. The thickness shear mode (TSM) acoustic wave sensor is the most popular acoustic wave device, generally known as Quartz Crystal Microbalance (QCM). Upon real time monitoring the variation of the sensor resonance frequency and motional resistance, or dissipation factor, and using acoustic impedance spectroscopy to model equivalent electric circuits the acoustic wave signal can be cleared up by distinguishing mass load from acoustic energy losses and charge induced parasite capacitive interferences due to the liquid viscosity and the presence of electrolytes, respectively. We have previously shown that this strategy was highly effective to assess both equilibrium and kinetic constants while giving some incites regarding conformational alterations and hydrophobicity of adsorbed proteins at the sensor surface.

In this work we extent the methodology and use acoustic impedance to screen binding of proteins to modified sensor surfaces. The goal is to establish specific correlations between acoustic energy losses and protein hydrophobicity as well as to directly monitor eventual conformational alterations upon protein binding.

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Poster Communications

Synthesis of palmitoyl xanthan for microencapsulation of chondrogenic cells by self-assembly process in physiological conditions

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Xanthan gum is an anionic extracellular polysaccharide produced by the bacterium *Xanthomonas campestris* and is known for its biodegradability, bio-adhesive and wound-healing properties [1]. Recently, it was applied as artificial matrix for cell encapsulation by ionic crosslinking [2]. Self-assembling properties of amphiphilic macromolecules in aqueous solutions can be used in the design of functional nanostructures. Based on these concepts, xanthan was conjugated with hydrophobic palmitoyl groups to obtain an amphiphilic system that can self-assemble under physiological conditions. The self-assembling properties of this amphiphilic system can be used as encapsulating matrix for cells.The characterization of the conjugates was performed by ^{1}H Nuclear Magnetic Resonance, Fourier Transform Infra-Red spectroscopy, Differential Scanning Calorimetry, Dynamic Light Scattering measurements, X-Ray Diffraction and Scanning Electron Microscopy. The microcapsule formation properties of palmitoyl xanthan were optimized and the viability and proliferation of encapsulated chondrogenic cell line were investigated by AlamarBlue® and DNA assays, respectively. Our studies revealed that the aqueous solutions of palmitoyl xanthan could form gels in physiologic ionic strength and pH by self-assembling into spherical hollow-capsules. The microcapsules were regular and stable in cell culture conditions with the capability of supporting the survival and biological function of encapsulated chondrocytes over prolonged time. The microcapsule forming property of palmitoyl-xanthan in physiological conditions makes this amphiphile a promising alternative to the conventional biomaterials employed in cell encapsulation to be applied in therapies by cell-delivery.

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Immobilization of antibodies in chitosan membranes for selective cell attachment

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One of the major goals in biomaterials science is to accurately control cellular interactions with implantable surfaces. [1] Covalent grafting of biomolecules is a strategy to improve the biocompatibility and bioactivity of materials. [2] In the present work we study the biological properties of chitosan, in which the surface was modified with an antibody for a specific type of cell. Using BS3 (Bis(Sulfosuccinimidyl) suberate), a crosslinker that will promote the covalent attachment of the antibody to the chitosan membranes we intend to functionalize the membranes. With this functionalized surfaces we aim to create smart surfaces that recruit a specific type of cell from a mixed cell population. Human adipose stem cells (ASCs) and SaOs-2 cells were seeded onto these surfaces to assess the biological consequences of such modifications.

It was found that after 1-2 hours incubation the cells that recognize the immobilized antibody were widely attached to the surface and significantly fewer cells were detected in unmodified membranes and in membranes with antibodies that are not recognized by the cells. The results of cell adhesion studies indicated that the antibody was successfully grafted to the membranes. After 24h incubation, ASCs were adhered and well spread on membranes functionalized with CD90, even in the absence of FBS. In opposition to unmodified membranes or functionalized with CD3 few cells adhered. Saos-2 adhesion for anti-CD90 modified membranes present also few cells indicating that only cell that recognize the antibody attach to the surface. As main conclusion, were able to produce surfaces that recruit and immobilize a cell target depending on the immobilized antibody.

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Photo-crosslinked peptide-carboxymethyl chitosan conjugates for higher cell proliferation and viability

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The study is focused on the synthesis and potential application of photo-crosslinked polysaccharide template functionalized with peptide sequences mimicking fibroin. The consecutive glycine (G)-alanine (A) sequence, was synthesized on the carboxymethyl chisosan (CMC) by using carbodiimide conjugation chemistry [1]. The obtained conjugates were stabilized by photo-crosslinking (photolabile sodium benzoate, irradiation at 240 nm) of casted films. The viability, proliferation and stimulation of biological functions of peptide-CMC were tested on osteogenic SaOs-2 cell line. SaOs-2 cultured on the different conjugates demonstrated higher expression of DNA in comparison with non-functionalized CMC and this tendency was kept for all studied periods. In addition, the quantity of DNA from cells exceeded or was close to the one measured for the cells cultured on tissue culture plate (TCP) between 7 and 21 days. The metabolism of SaOs-2 on the peptide-CMC conjugates was much higher than the parent polysaccharide. The GA and GAG sequences gave highest metabolic activities at the 14th and 21st day of culture. The alkaline phosphatase (ALP) activity of conjugates was higher than CMC or TCP in all time points. The highest activity was observed with GA sequence and this was more pronounced at the 21st and 28th days. The alizarin test was conducted for observing mineralization of used biomaterials. The staining was higher on the peptide-polysaccharides than on the CMC and TCP samples. These results showed higher viability and proliferation of cells on materials with longer peptide sequences as well as improved osteogenic functions when compared to the native polysaccharide or TCP.

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Preparation and characterization of Bioactive glass-ceramic nanoparticles for biomedical applications

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Since the first bioactive glass developed by Hench in 1971, bioactive glasses have been widely used for bone tissue engineering [1]. Its high bioactivity makes the materials bond rapidly to both hard and soft tissues without fibrous encapsulation, and enhances bone formation. Bioactive glasses have unique characteristics that make them an important material in the field of bone regeneration, namely its rapid rate of surface reaction promoting direct attachment to the bone via a chemical bond, relatively low sintering temperatures that are important to the ceramic particles bonding and micropores filling, and the wide range of possible compositions, leading to different chemical properties and rate of bonding with tissues, allowing more specific clinical applications to nano sized particles.

This work aims to investigate the morphology and bioactive capability of BG-NPs by considering the effects of different compositions, pH and thermal treatments on the in vitro bioactivity of BG-NPs. Bioactive glass nanoparticles (BG-NPs), based on both ternary (SiO_2-CaO-P_2O_5) and binary (SiO_2-CaO) systems, were prepared via a sol-gel method based on a previous work [2]. The P-free system applied to the production of BG-NPs is proposed, in order to evaluate the effect of suppressing this component, in the bioactivity capability of the materials. The bioactive character of the BG-NPs was accessed *in vitro* by analyzing the capability for apatite formation onto the surface after being immersed in simulated body fluid (SBF). The morphology, composition and bioactivity potential of the BG-NPs were studied using FTIR, XRD, EDX and SEM.

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Uncovering Epidermal Stem Cells from Adult Keratinocyte Cultures

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Current treatments for massive skin loss are still in their infancy and Skin Tissue Engineering (TE) remains as the most promising approach to meet the demands on design best quality skin. The long-term function of current skin equivalents is often limited by the terminal differentiation of the grafted keratinocytes. Thus, the use of Epidermal Stem Cells (EpSCs) appears as a powerful tool providing an active source of biological material.

EpSCs isolation difficulty remains, due to the lack of well-determined approaches and markers. This work integrates an assemblage of strategies to be pursued in order to accomplish enrichment of this multipotent fraction.

After isolating human primary keratinocytes (hKC) from human adult skin, after informed patient consent, Rho-Associated Protein Kinase (Rock) Inhibitor Y- 27632 was firstly administered to freshly hKC cultures to increase EpSC number [1]. Consecutive selective methods such as rapid adherence to β1-integrin ligand in collagen type IV [2] and immunomagnetic separation methods were then performed to establish populations based in the α6/CD71 expression. In order to study the outcome of the proposed strategies, their clonogenic capacity, growth rate and long-term proliferation were compared to non-EpSCs enriched populations. CFUs assay, flow cytometry analysis and immunocytochemistry were then performed, focusing on the effect of the treatments, over expression rate of early epidermal markers keratins 19/5/14 and correlated with α6/CD71 sub-populations. Methodologies were refined based on the analysis of a significant number of skin samples.

The methodology presented in this work indulges the boosting of EpSCs in hKC culture, with a consecutive purification and separation from hKC bulk.

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Development of an injectable system based on elastin-like polymer microparticles for tissue engineering applications

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Elastin-Like Polymers (ELP) are genetically engineered polymers that can be tailored in order to fit the requirements of specific applications [1]. A RGD-containing ELP was used for fabrication of microparticles by an innovative and affordable method based on the superhydrophobicity of modified polystyrene surfaces. Two different microparticles types were prepared, varying the crosslinking extent. The influence of the crosslinking extent was tested using an osteoblast-like cell line (SaOs-2) by performing viability, proliferation and osteogenic expression tests, as well as by the assessment of the morphology and cell distribution on the particles.

This work aimed the *in vitro* formation of cell-induced microparticles aggregates in order to extrapolate the formation of *in situ*-forming scaffolds in case of injection of the particles into a defect.

ELP particles have been successfully obtained by deposition of polymeric solution in superhydrophobic surfaces; two different crosslinking extents – of 22% and 60% - were achieved controlling the time of exposure to the crosslinker. Those affected the swelling behavior as well as the mechanical properties of the particles, which were proven to affect cell behavior [2]. SaOs-2 viability, morphology, osteogenic expression, spatial distribution and ability to bind particles together were also dependent on the physicochemical properties of the microparticles: the higher crosslinking extent condition proved to be the most favorable condition for cell growth and ability to form cell-induced aggregated structures, turning it into a possibly interesting application for, e.g., bone tissue engineering applications.

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Flow perfusion can stimulate the chondrogenic differentiation of human mesenchymal stem cells cultured onto chitosan-based scaffolds

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Native articular cartilage is subjected to synovial fluid flow during normal joint function. Thus, it is believed that the morphogenesis of articular cartilage may be positively regulated by the application of similar stimulation in vitro. In the present work, the effect of medium flow perfusion over the chondrogenic differentiation of human bone marrow derived mesenchymal stem cells (hBMSCs) is studied. We aim at conclude if the shear stress caused by the medium perfusion trough the constructs is able of positively regulate the differentiation process.

Human BMSCs were seeded statically onto the fiber meshes scaffolds, consisting of a blend of 50/50 chitosan and Poly(Butylene Terephtalate Adipate)–CPBTA. The constructs were cultured in a flow perfusion bioreactor for 28 days. An enhanced ECM deposition and collagen type II production was observed in bioreactor samples, when compared to static controls. The ECM accumulation in those samples is lower than in those cultured in the bioreactor, and there is a significantly higher expression of collagen type I, indicating a tendency for the production of fibrocartilage in those conditions. Our results are consistent with other studies in the literature that showed that dynamic shear, in the absence of hydrostatic pressure gradients, induced matrix protein transcription [1]. Moreover, it was observed that dynamic shear increased extracellular matrix biosynthesis, preferentially collagen II synthesis [2].

In summary, it was shown that the flow induced shear stress has a positive effect on the chondrogenic differentiation of hBMSCs being interesting in the context of developing functional articular cartilage regeneration.

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Functionalized multi supportive structures aimed for spinal cord injury repair

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Spinal cord injury (SCI) represents a significant world health problem. It has been reported that the world annual incidence of SCI varies between 11.5 and 57.8 cases per million. Therefore it is urgent to develop novel strategies that can specifically target this problem. In this context, the objective of the present work was to develop a new range of 3D tubular structures aimed at inducing the regeneration within SCI sites. Up to six different 3D tubular structures were initially developed by rapid prototyping - 3D bioplotting – based on a biodegradable blend of starch with poly-ε-caprolactone (SPCL). These structures were then further complemented by injecting Gellan Gum, a polyssacharide based hydrogel, in the central area of structures. Their mechanical properties were then assessed by DMA, under both dry and wet conditions, and their morphologies/porosities analysed by micro-CT and SEM. Biological evaluation was carried out for determining their cytotoxicity, using both MEM extraction and MTS tests, as well as by encapsulation of an oligodendrocyte like cell (M03-13 cell line) within the hydrogel phase. Finally the *in vivo* regenerative potential of the developed structures was access in a hemisection SCI model in Wistar rats. After two months their locomotory and sensory behaviour was accessed. The histomorphometric analysis showed a fully interconnected network of pores with porosity ranging from 70%-85%. Scaffolds presented a compressive modulus ranging from 17.4 to 62.0 MPa and 4.42 to 27.4 MPa under dry and wet conditions, respectively. Cytotoxicity assays revealed that the hybrid SPCL/Gellan Gum scaffolds were non cytotoxic as they did not cause major alterations on cell morphology, proliferation and metabolic viability. Moreover, cell encapsulation assays showed that the hybrid scaffolds could support the *in vitro* culture of oligodendrocyte like cells. Finally, preliminary *in vivo* assays revealed that the Hybrid SPCL/gellan gum scaffolds was well integrated within the spinal cord environment and did not trigger an inflammatory response by the host. Further work will be focus now on the functionalization and neuronal guidance capability of the gellan gum based hydrogel.

Evaluation of the effect of cryopreservation over the functionality of bone-generating cell/tissue constructs

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The tissue engineering community is becoming increasingly concerned with the issue of preserving and storing "living" biomaterials. Cryopreservation seems to be the most tenable option for solving this problem since it is based on the principle that chemical, biological and physical processes are effectively "suspended" at cryogenic temperatures [1].

The aim of this work was to study the effect of cryopreservation over the functionality of both 2D and 3D tissue engineered constructs and to compare the cellular survival and metabolic activity of cells seeded, cultured and cryopreserved on them. Apart from evaluating the effect of cryopreservation on the cells cultured onto polymeric scaffolds, this work also intended to evaluate its effect over the scaffold material itself.

For this, several 3D porous scaffolds and 2D nonporous discs made of a starch-poly(caprolactone) polymer blend (SPCL) were seeded with goat bone marrow stem cells (GBMSCs) and cryopreserved for 7 days in liquid nitrogen. After this period, the samples were analyzed and compared to non-cryopreserved samples.

The obtained results suggest that it is possible to maintain cell viability and scaffolds properties upon cryopreservation of tissue engineered constructs based on SPCL scaffolds and GBMSCs using standard cryopreservation methods. Also, this study tends to suggest that the greater porosity and interconnectivity of scaffolds favor the survival of construct's cellular content during cryopreservation processes. These findings indicate that it might be possible to prepare off-the-shelf engineered tissue substitutes and preserve them in order to be immediately available upon patient's need.

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Osteogenic Cell Sheet Engineering for Bone Tissue Engineering Purposes

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Bone Tissue Engineering (TE) strategies are typically based on the use of scaffolds in combination with osteogenic cells. This has, however, failed to produce the desired results due to issues such as the immunogenicity of the biomaterials used, and cell necrosis at the bulk of the scaffold related to deficient oxygen and nutrients diffusion. The use of cell sheet (CS) engineering is herein proposed as a way to overcome some of these obstacles. In a first stage the potential of a single osteogenic CS to induce bone formation *in vivo* was tested. Osteogenic CSs were fabricated by culturing rat bone marrow cells in thermo-responsive culture dishes. The CSs were recovered from the dishes using a low temperature treatment and subcutaneously implanted in nude mice. New bone formation was verified from day 7 post-transplantation using x-ray, μ-CT and histology. The presence of a vascularized marrow and osteocytes supports the conclusion that the tested CSs induced the formation of mature bone tissue. In a second stage, the potential of adding endothelial cells to the osteogenic CSs to improve the vascularization of the newly formed bone was assessed. Human umbilical vein endothelial cells (HUVECs) were seeded between two osteogenic CSs and implanted in the same model. Histological analysis post-implantation showed a higher quantity of mineralized tissue in the samples with HUVECs. Furthermore, perfused vessels, positive for human CD31, confirmed the participation of HUVECs in the bone tissue neo-vascularization. These results therefore validate the great potentiality of CS engineering to be used in bone TE.

Adipose Tissue-Derived SSEA-4 Subpopulation Demonstrates Promising Features for Bone Tissue Engineering

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Human Adipose-Derived Stem Cells (hASCs), an underappreciated source of stem cells, might comprise a new cell poll for tissue engineering (TE) applications, including bone TE [1]. Considering the fact that SSEA-4 is a marker of pluripotency, the endothelial and osteogenic differentiation potential of the SSEA-4$^+$ subpopulation within the hASCs (SSEA-4$^+$hASCs) was evaluated, aiming at developing a new strategy for promoting vascularisation of bone TE constructs.

SSEA-4$^+$hASCs were isolated using an immunomagnetic sorting technique and were cultured either in basal medium, in EGM-2 MV (endothelial growth medium) or osteogenic medium. Cells cultured in EGM-2 MV formed endothelial-like colonies characterized a typical endothelial cobblestone morphology and expressing endothelial specific markers (CD31, CD34, CD105, von Willebrand factor) as confirmed by real time RT-PCR, immunofluorescence and flow cytometry The endothelial character of these was also confirmed by their ability to incorporate acetylated low-density lipoprotein and to form of capillary-like structures when seeded on Matrigel. However, when SSEA-4$^+$hASCs were cultured in basal medium, they displayed a fibroblastic-like morphology and exhibited a mesenchymal surface markers profile (>90% CD90$^+$, CD73$^+$ and CD105$^+$). An upregulation of osteogeninc-related markers (osteopontin and osteocalcin) was observed both at molecular level and protein expression levels, by culturing these cells in osteogenic differentiation medium. Matrix mineralization detected by Alizarin Red staining confirmed the successful osteogeninc differentiation of the SSEA-4$^+$ hASCs. This work demonstrates that adipose tissue allows selection, *in vitro* culture and expansion of cells with high angiogenic and osteogenic potential. Thus, these cells could provide a potential cellular source for engineering vascularized bone tissue.

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Poly(D,L-lactic acid)/Bioglass ® membranes for biomedical applications.

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In guided bone regeneration, membranes are been used as a physical barrier to create a secluded space around the bone defects by preventing the invasion of soft tissue into the defect space, thus allowing the missing bone tissues to be regenerated [1,2]. The two sides of the membranes are in contact with distinct biological environments in which one faces a region in which osteointegration should be ideally promoted. Biocompatible and biodegradable composite membranes were produced by combining poly(D,L-lactic acid), PDLLA, and Bioglass® particles featuring an asymmetric bioactivity and a good integration between the polymeric and inorganic fractions. The asymmetric distribution of the osteoconductive particles is produced during the processing of the membrane using a solvent casting methodology: only the inorganic-rich face could promote the deposition of bone-like apatite after immersing the composite membrane in simulated body fluid for 2 days. The microstructure and surface properties were characterized using Scanning Electron Microscopy, and Fourier Transform Infrared Spectroscopy. The mechanical properties of the membranes were evaluated by Dynamic Mechanical Analysis by analysing the viscoelastic properties and the glass transition of the samples in both dry and wet states. A clear plasticization effect of water could be detected but the composite membranes were founded to be stiffer at 37 ºC as compared with the pure polymer. The method of production used in this composite system allows to obtain membranes that exhibit osteoconductive properties in just one of the faces that could find applications in guided bone regeneration and regenerative medicine applications.

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Chitosan/soy-based membranes as wound dressing devices

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Chitosan-based biomaterials proved to have promising characteristics for wound dressing and skin regeneration [1]. In the context of developing new natural-based biomaterials for these applications, chitosan and soybean-based biomaterials were proposed. These materials were shown to be non cytotoxic and to impair human leukocytes activation *in vitro* [2]. Thus the goal of this study was to evaluate the *in vivo* performance of chitosan/soy-based membranes in the regeneration of partial thickness skin wounds. Excisional skin wounds were created on the backs of rats and the healing capacity of chitosan/soy-based membranes was assessed after 1 and 2 weeks. To promote impaired wound healing all rats were injected with a steroid. After one week, membrane-dressed wounds showed less granulation tissue formation and the wounds margins were thinner than in untreated wounds (negative control). Furthermore, in comparison to the positive control (Eppigard®), the chitosan/soy-based membranes were also able to reduce the wound size and the thickness of the wound limits. A similar trend was observed 2 weeks after the dressing; the membrane-dressed wounds were almost closed, although with some contraction, and with almost no granulation tissue. For both time periods there were no signs of infection. The observed re-epithelialization and engraftment between the regenerated tissue and the original skin reinforced the suitability of the proposed chitosan/soy-based membranes to be used in skin wound dressing.

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Nanoparticle-based Platelet Lysate Release Systems for Enhanced Proliferation of Human Adipose Serived Stem Cells in Combinatory Tissue Engineering Strategies

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Recently, the delivery of growth factors (GFs) through controlled release systems is one of the top approaches for combined tissue engineering (TE) and regenerative medicine strategies. Polysaccharides have been motif of interest for their versatility and superior functional properties and can be used to design new protein delivery systems since they are capable of preserving the bioactive conformation and even enhancing the potency of the proteins. In this work, a polyelectrolyte complex (PEC) based on the electrostatic interaction of two oppositely charged polysaccharides, chitosan (CH) and chondroitin sulfate (CS) is proposed for the encapsulation of proteins, specifically platelet lysate (PL). PL is a promising source of GFs due to its autologous nature and rich composition, leading to synergistic GF actions [1]. By a harmless and quick procedure for the entrapped proteins, spherical nanoparticles (NPs) slightly smaller than 200 nm were developed. The PL-loaded NPs were cultured with human adipose derived stem cells (hASCs) and it was observed a positive influence over cell viability and proliferation. The NPs were then included in a three-dimensional polyD,L lactic acid (PDLLA) scaffold to address the possibility of creating a multifunctional scaffold able to stimulate the proliferation and differentiation of seeded cells. The PDLLA-NPs hybrid structure shows the potential to be used as multifunctional scaffold for TE applications due to its porosity, interconnectivity and the possible different protein release rates, which can be achieved and designed with different ratios of NPs incorporation within the PDLLA scaffolds.

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Bioinspired methodology to fabricate hydrogel spheres for biomedical applications using superhydrophobic substrates

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Hydrogel spheres have aroused great attentions for many years because of their applications in biomedicine, biotechnology or biochemistry. They can present excellent biocompatibility, contain large quantities of water and surface area, and may be assembled into different shapes. Many methods have been developed for preparing hydrogels, such as cross-linking by the action of temperature; light or electron beam irradiation; free radical or electrochemical polymerization; microemulsion polymerization; or by induction of physical cross-links. In most of the cases hydrogel spheres are produced in a liquid environment, such as by precipitation in a coagulation bath or by emulsion. During the formation of the hydrogel spheres a fraction of the molecules that are initially in the liquid phase may be lost. Therefore, the encapsulation of cells, proteins or other molecules will be never totally efficient in such "wet" methods. In this contribution we propose a new method that enables to encapsulate living cells and molecule with high efficiency and in mild conditions [1]. Inspired by water rolling on the lotus leaf, a completely new fabrication process of hydrogel spheres using a dry method is developed, in which liquid droplets, that acquire a spherical shape when suspended over a superhydrophobic substrate, harden into a hydrogel form without being in contact with any other liquid media. Proofs of principle are given in this work, including for the production of particles for cell expansion, and spheres for cell encapsulation or drug delivery, using hydrogels of chitosan or alginate.

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Self assembling nanogels

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Polymeric nanogels - also referred to as hydrogel nanoparticles, macromolecular micelles or polymeric nanoparticles - are emerging as promising drug carriers for therapeutic applications. These nanostructures hold versatility and properties suitable for the delivery of bioactive molecules, namely biopharmaceuticals. The polymer and the production methodology used are fundamental options. These systems may be obtained by incorporation of targeting moieties, detectable probes and/or degradable bonds allowing a controlled release in the physiologic environment which lead to smart systems reactive to physiologic stimuli, etc. A particular challenge in this field is the development of preparation procedures avoiding the use of organic solvents or surfactants.

The production of self-assembled nanogels made of polysaccharides is thus a promising approach for the development of delivery systems. Amphiphilic molecules, obtained from polysaccharides - such as dextrin, chitosan or glycol-chitosan, mannan, hyaluronic acid - self-assemble in aqueous medium, originating nanoparticulate material holding hydrophobic cores which may hold, carry and eventually release pharmaceuticals, including biopharmaceuticals. The production and characterization of these materials, the study of the interaction with therapeutic proteins (IL10) and low molecular weight hydrophobic drugs, a comprehensive characterization of biocompatibility, including cytotoxicity, intracellular trafficking, biodistribution, are among the completed tasks in this field. The development of adjuvant formulations for vaccination and delivery systems is the final goal.

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Membranome analysis of benzalkonium chloride adapted Planktonic and Biofilm cells

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Adaptive resistance to antimicrobials has been widely reported trough phenotypic characterization and proteomic analysis. Concerning biofilm adaptation, the response of biofilm-entrapped cells to chemical stress conditions is not yet well-studied. Membrane proteins play key roles in cell life cycles and are central in resistance mechanisms. The outer membrane (OMP) is an important compartment where metabolites circulated, constituting more than half of all known drug targets.

This work aimed to examine whether antimicrobial adaptation of planktonic and sessile *Pseudomonas aeruginosa* to benzalkonium chloride (BC) could induce a membranome alteration. This was inspected by proteome alterations of the OMP of planktonic and biofilm cells. Adaptation was attained by continuous exposure to increasing doses of the antimicrobial. Planktonic adaptive resistance was induced by subculturing *P. aeruginosa* in TSB supplemented with increasing concentrations of BC. Biofilms were formed for 24h being after submitted to maturation in the presence of BC. Biofilm and planktonic non- and adapted cells were collected and the OMP extracted. Protein patterns were analysed by 2D and gels by the SameSpot software.

Results showed that the protein contents of biofilm cells are differentiated from the suspended counterparts. The 2D pattern of adapted biofilm cells is also different from the adapted planktonic bacteria, revealing that the response to BC is different if cells are free floating or in sessile state.

This study highlighted that there might be different membranome regulation when bacteria are submitted to chemical adaptation. This particular response to the environment can be one of the causes of the well-known biofilm resistance phenotype.

Presence of extracellular DNA in *Candida albicans* biofilm matrix and its role in biofilm structure and antifungal susceptibility

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Biofilms are structurally complex microconsortia of surface adhering cells embedded within an extracellular matrix (ECM) composed of substances produced and secreted by cells or derived from cell lysis. One of the recently discovered bacterial biofilms ECM components is the extracellular DNA (eDNA). Although the investigation on eDNA in fungal biofilms is scarce, preliminary studies suggest that eDNA may play a role in biofilms formed by the opportunistic fungal pathogen *Candida albicans*. Thus, the present study aimed at determining the eDNA content of *C. albicans* SC5314 biofilm ECM and the effect of DNase I treatment on biofilm formation and biofilm cells susceptibility to antifungals, as indicators of the role of eDNA in *C. albicans* biofilms.

Results from our experiments showed that the ECM of *C. albicans* biofilms formed under conditions of flow for 48 h contained 3045.4 ± 227.3 ng eDNA/mg of protein. Additionally, using a microtiter plate model, we observed that different DNase treatments (0.02 - 2 mg/ml) did not affect further biofilm development by *C. albicans* adherent cells. However, DNase (> 0.03 mg/ml) promoted a general biomass reduction on *C. albicans* preformed biofilms. Finally, DNase (0.13 mg/ml) did not change *C. albicans* biofilm cells susceptibility to fluconazole, but increased their susceptibility to amphotericin B and caspofungin, as indicated by the lower SMIC compared to biofilms grown without DNase.

This work presents evidence for the role of eDNA in *C. albicans* biofilm integrity and antifungal resistance consistent with eDNA being a key element of the ECM.

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Fungal silver nanoparticles

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Due to the outbreak of infectious diseases caused by different pathogenic microorganisms and the development of drug resistance, nanoscale materials have emerged up as novel antimicrobial agents. Nanomaterials can be synthesized by conventional chemical methods, but most of them are regarded as highly environmental cost. Generally, the chemical methods are low-cost for high volume; however, their drawbacks include contamination from precursor chemicals, use of toxic solvents, and generation of hazardous by-products. Development of reliable and eco-friendly processes for synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. Also the importance of bactericidal nanomaterials study due to the increase in new resistant strains of bacteria and fungi against most potent antibiotics has promoted research in the well known activity of silver ions and silver-based compounds [1]. For this reason, there is an essential need to develop environmentally benign procedures for synthesis of silver nanoparticles for commercialization purposes.

In relation to other microorganisms fungi present key characteristics such as tolerance and metal bioaccumulation abilities that are advantageous for production of nanoparticles. In this study, silver nanoparticles were synthesised extracellularly from silver nitrate using the fungi supplied by Micoteca da Universidade do Minho (MUM) fungal culture collection, and the morphology of the nanoparticles was characterised The potential to manipulate key parameters, which control growth and other cellular activities, to achieve an optimised production of nanoparticles were also investigated. In addition, a preliminary study was performed to assess the anti-fungal silver nanoparticles activity against bacteria.

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Genome characterization of two valuable therapeutic bacteriophages against Salmonella and Campylobacter

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Salmonella and *Campylobacter* are recognized worldwide as the major foodborne pathogens responsible for human gastrointestinal diseases. The increased resistance of bacteria to antibiotics has encouraged the development of alternatives to control bacterial pathogens. Bacteriophages (phages), as natural predators of bacteria, are considered an appealing option. We report herein the isolation and genome characterisation of two *Myoviridae* broad lytic spectra *Campylobacter* (vB_CcoM-IBB35) and *Salmonella* phages (PVP-SE1) with high potential for therapy. The majority of genes of vB_CcoM-IBB35 are unique although homology exists with members of the *Teequatrovirinae.* Unique genes involved in pathogenesis, carbohydrate and amino acid metabolism were also observed along with several incidences of gene duplications, split genes with intein and introns and “insertion-like sequences”.

From the 244 genes found in PVP-SE1, approximately 46% encode unique proteins and only 22.1% exhibited homology with known proteins. The genome sequence presents high homology (145 gene encoding proteins) with the *E.coli* bacteriophage rV5, both unrelated to any other known phage, which might suggest that they belong to a new phage genetic group. As conclusion, one can argue that the genomic characterization of both phages did not reveal any factor which could preclude its therapeutic use.

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ASPM is an oncoprotein and activates EGFR

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ASPM is a protein required for the correct assembly and function of microtubule spindles. It participates in human brain development as well and is overexpressed in all tumours studied so far. We are interested in further characterizing ASPM function as it is unclear why it affects brain growth and is related to cancer. This prompted us to isolate ASPM-binding proteins. We found that EGFR (Epidermal Growth Factor Receptor), a protein highly involved in cancer, would bind ASPM. Furthermore, ASPM promotes activation of EGFR by phosphorylation resulting in a more active MAP kinase pathway. Our findings explain the involvement of ASPM in cancer, set ASPM as a new therapeutic target, and demonstrate a novel activity of the protein not related to mitosis.

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NovaFlow – Novel applications of a state-of-the-art oscillatory flow platform: hydroxyapatite production and its use in bone extracellular matrix growth

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Hydroxyapatite (HAp) $Ca_{10}(PO_4)_6(OH)_2$ is of significant interest in biomedical engineering due to its exceptional biocompatibility, bioactivity and osteoconductivity properties [1].

In this context, the present project aims at synthesizing HAp crystals with a carefully controlled size, with a controlled and narrow size distribution and with a high purity. Thus HAp crystals shall be produced with a high specific surface area, i.e. small crystals, and a high biocompatibility, making them suitable for application in bone substitution.

The work should start by the characterization of HAp precipitation process, namely by the definition of the optimal operation conditions and the modelling of the process. First, the study will be conducted in a stirred tank. Once the system characterized, HAp precipitation will be carried out in a novel OFR developed by CEB-UMinho [2]. In fact, the OFR appears as a good candidate to promote ideal conditions, in particular in terms of micromixing, for the controllability of HAp particles properties. The effect of some additives with well defined roles in HAp precipitation will also be investigated. In parallel, the biocompatibility of the products developed will be evaluated, by determining the cytoxicity using cell lines (osteoblasts). Finally, the novel OFR will be tested for bone related cells culture, both in the absence and in the presence of HAp crystals.

Initial experiments were performed in a 1L stirred tank, mixing a saturated solution of calcium with a solution of phosphate using different Ca/P molar ratios always at T=37ºC. After process optimization, a suspension of stoichiometric HAp particles with pH close to 7 was obtained for Ca/P=1.29. The particles formed possess a rod-like shape with dimensions of about 20 nanometers thick and 100 nanometers long, and proved to be high crystalline. In addition, HAp crystals revealed the tendency to aggregate in solution and had a narrow size distribution with a mean particle size of about 128 nm.

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Innovative uses for microbial products: bacterial protein azurin as a new anti-cancer drug candidate

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Azurin is a low molecular weight protein produced by the bacteria *Pseudomonas aeruginosa*. Besides its described function as a redox partner in electron transfer reactions, azurin can act as an anticancer agent. It has been demonstrated that azurin can enter preferentially into cancer cells and forms a complex with the tumor suppressor p53, stabilizing it and inducing apoptosis [1]. Azurin also allows in vivo cancer regression in immunodeficient nude mice [1]. Azurin presents an immunoglobulin-like fold and a large exposed hydrophobic patch. These observations allowed the discovery that one of the most important properties of azurin is its ability to mediate high-affinity interactions with unrelated proteins relevant in cancer, conferring on it the property of a natural scaffold for therapeutic purposes. This effect has been already proven for the receptor tyrosine kinase EphB2-mediated cell signaling, since azurin, due to its interaction with this receptor, prevents its binding to the ligand ephrinB2 [2]. The present work has the intention to extend the azurin studies to the cell adhesion molecules, with an emphasis on P-cadherin. P-cadherin is frequently overexpressed in high-grade invasive breast basal-like carcinomas and it is a poor prognosis marker in breast cancer. Due to the structure similarities of azurin and the extracellular cadherin domains, we hypothesized that azurin could bind to P-cadherin and inhibit its effects. Protein-protein docking results and SPR experiments have validated this approach. Results of the ongoing work conducted to elucidate the cellular effects of treating cell models of high P-cadherin expression with azurin will be presented. Thus, we hypothesized that azurin could be a scaffold against P-cadherin in breast cancer cells, antagonizing its pro-invasive effects being an interesting new therapeutic tool in the treatment of this specific type of breast cancer.

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Insights into the transcription regulatory network controlling the multidrug resistance gene *FLR1*: a systems biology approach

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Multidrug resistance (MDR) often results from the activation of drug efflux pumps, many times controlled at the transcriptional level. Functional genomics tools have been used to characterize the Drug:H^+ Antiporter (DHA) family in the eukaryotic model system *Saccharomyces cerevisiae* [1]. The complex transcriptional control of these genes has also been on the focus of our research, guided by the YEASTRACT database [2, 3].

In this study, the transcriptional activation of *FLR1*, encoding a DHA transporter, in response to stress induced by the fungicide mancozeb was scrutinized. Previous studies in the context of environmental genomics [4], contributed to understand the proteome-wide response [5] and tolerance mechanisms [6] towards mancozeb. *FLR1* up-regulation was shown to depend on the integrated action of four transcription factors [7, 8, 9]. More recently, a mathematical model describing the *FLR1* regulatory network was built and its response to mancozeb stress in different genetic backgrounds was simulated, using the Genetic Network Analyser software. This approach allowed the identification of essential features of the transition from unstressed to fungicide stressed cells and to make new predictions on the dynamic behaviour of the system, which were validated experimentally [11]. In particular, the role of Pdr3 in *FLR1* regulation was further scrutinized and the inter-dependent role of Yap1 and Yrr1 in the regulation of *PDR3* and *RPN4* was brought to light. The comparison between experimental and simulated data highlighted the need to consider the participation of a fifth, still unidentified, transcription factor to fully explain the network's behaviour.

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Molecular strategies employed by *Burkholderia cenocepacia* to adapt and persist in the airways of cystic fibrosis patients, revealed by genome-wide approaches

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The epidemiological survey of *Burkholderia cepacia* complex (Bcc) bacteria involved in severe respiratory infection among the Portuguese cystic fibrosis (CF) population under surveillance at Santa Maria Hospital, in Lisbon, has been carried out by our research group. This study covers isolates of *B. cenocepacia*, *B. cepacia*, *B. multivorans* and *B. stabilis*, obtained since 1995 [1,2,3]. The ribopattern/ multilocus sequence typing (MLST) profiles of sequential isolates of the same species retrieved from a particular patient during chronic infection indicated that they are clonal variants exhibiting variable phenotypes relevant in Bcc persistence and virulence. In particular, their antibiotic susceptibility profiles varied during infection [4]. Chronic respiratory infections caused by these bacteria are, in general, characterized by low responsiveness to antibiotic therapy and rapid reduction of lung function. Since antibiotic resistance is an important facet of management of these infections, the expression programs of a *B. cenocepacia* isolate that presumably started the infection and of a clonal variant highly resistant to multiple antimicrobials, obtained after 3 years of persistent infection and intravenous antimicrobial therapy, were compared. This study involved quantitative proteomics, based on two-dimensional Difference Gel Electrophoresis (2D-DIGE), and a pangenomic microarray analysis. Results from these genomic expression analyses provided insights into the molecular strategies employed by *B. cenocepacia* to deal with the stressing conditions to which they are exposed in the CF lung. These include aggressive antibiotic therapy which led to the selection of variants with increased resistance levels to multiple antimicrobials. A computational analysis of B. cenocepacia J2315 (ET12 lineage) genome identified a trimeric autotransporter adhesin cluster, unique to this epidemic lineage. Knock-out mutants were assayed for adherence to extracellular matrix proteins, biofilm formation, serum resistance, hemagglutination ability and virulence against *Galleria mellonella* as infection model. Results show that this adhesin cluster represents a novel virulence locus that plays an important role in cellular adhesion and virulence [5].

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Evaluation of the transfection efficiency of retinal cells by chitosan nanoparticles

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Gene therapy has relied so far in viral vectors. These have a higher transfection efficiency, which has not been matched by non-viral vectors, whether they are liposomes or polymers. Efforts into improving the efficiency have included several approaches, with one of these being chemical modifications of polymers in order to optimize nucleic acid incorporation, release profile and protection from degradation.

We have tested nanoparticles of either chitosan-DNA and thiolated chitosan-DNA for their transfection efficiency in retinal cells. In order to promote the release of the nucleic acid within the cytoplasm and hence facilitate its entry into the nucleus, we have chemically modified chitosan by inserting a disulfide bond that can be cleaved cytoplasmatically in the presence of gluthatione. We have produced chitosan and thiolated-chitosan nanoparticles with defined sizes and these were used to deliver a GFP-expressing plasmid to cells from the retinal pigmented epithelium.

Our results show that chitosan was effectively modified to incorporate a disulfide bond. The transfection efficiency of chitosan and thiolated chitosan varied according to the cell line used, but showed no difference between the unmodified and the modified form. The method of administration of the nanoparticles has shown to greatly influence the transfection efficiency.

Ongoing efforts to elucidate the lack of improvement of the transfection efficiency by thiolation of the chitosan include fluorophore-tracking of the pathway undertaken by the nanoparticles within the cell and alternative methods of administration of the particles to the cells.

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Sucrose prevents protein fibrillation through compaction of the native state

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In many neural diseases one of the main hallmarks is the aberrant aggregation of proteins and most of the drugs developed against these diseases intend to inhibit aggregation or protect targeted cells from death.

A large number of strategies is being developed to unveil the submolecular and folding mechanisms involved in aggregation pathways and to interfere with it. There has been shown that sugars and other osmolytes interfere with the folding and fibrillation of proteins by stabilizing them thermodynamically.

To study the effect of the osmolyte sucrose on protein fibrillation, the S6 ribossomal protein of *Thermus thermophilus* was chosen. In this study the effect of sucrose on S6 fibrillation was evaluated, and the molecular mechanisms involved on the inhibition of protein fibrillation was characterized. Sucrose prevents protein fibrillation compaction of native states.

Mouse prion protein was then used to know if the preventive effect of sucrose on protein fibrillation would be relevant and a general mechanism acting on clinical relevant proteins. Misfolding and aggregation of prion protein is the cause for the neurological degenerative diseases called spongiform encefalopathies. Our results prove the preventive role of sucrose on the fibrillation of the prion protein.

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Limb molecular clock´s dependence on the major limb signaling centers

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Chick limb develops as a mass of mesenchymal cells encompassed into an ectodermal jacket that expand and transform along the proximal-distal (PD), anterior-posterior (AP) and dorsal-ventral axes. Fibroblast growth factors (FGFs) produced in the apical ectodermal ridge (AER) and Sonic Hedgehog (SHH) produced in the zone of polarizing activity (ZPA) establish the PD and AP axis, respectively. In 2007, we reported the Notch target gene *hairy2*, to perform 6 hour cyclic expression in limb chondrogenic precursor cells (*Pascoal et al., J Mol Biol 2007*), resembling the expression of clock genes during somitogenesis unveiling a parallelism between the processes of limb and trunk development. At present we are exploring the role of the major limb signaling centers AER and ZPA in the regulation of limb molecular clock gene, *hairy2*, expression. By in-ovo ablation and bead implantation experiments we overexpressed and downregulated FGF8 and SHH in *hairy2* positive (ZPA and the distal limb mesenchyme) and negative (anterior and posterior limb mesenchyme) limb domains. Our results show that FGF8 could induce *hairy2* in all the tested domains except the anterior limb mesenchyme, whereas SHH beads implanted in this domain is able to induce ectopic hairy2 expression in a concentration dependent manner. Furthermore, we performed a series of AER and or ZPA ablation experiments to analyze whether this difference in the induction of ectopic *hairy2* expression is due to the absence of SHH in the anterior limb mesenchyme. Our results point to the necessarily of both the AER derived FGFs and the ZPA derived SHH for the normal expression of *hairy2* in the limb distal mesenchyme. In our ongoing experiments we are analyzing the intra cellular pathways responsible for the aforementioned interactions. Moreover, we are testing the existence of a limb determination front that would establish limb bone elements size, while they are being laid down and assessing the precise role of Notch signaling during this process. Our data suggest that this determination front can be underlined by the counteracting gradients of FGF and RA, further reinforcing the parallelisms with the somitogenesis process.

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Probing the prion protein interactions on cell surface by FRET

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Prion Proteins (PrP) are cell surface proteins which are endogenous to brain cells and several other cell types in mammals. The conversion of the normal membrane PrP (PrP^{C}) into a pathogenic scrapie conformation (PrP^{Sc}) in mammalian neurons is responsible for Transmissible Spongiform Encephalopaties (TSE). PrP^{Sc} templates its conformation to resident PrP^{C} to generate new PrP^{Sc} but the nature of the PrP^{Sc}-PrP^{C} complex is still unresolved.

The aim of our work is to probe conformational changes and the aggregation of PrP by Fluorescence Resonance Energy Transfer (FRET). FRET has been selected for the study of PrP interactions due to its high spatial resolution. We have engineered separate fusions of mouse PrP to CFP (Cyan Fluorescent Protein) and to YFP (Yellow Fluorescence Protein), PrP-CFP-GPI and PrP-YFP-GPI, respectively. Both constructions include a peptide signal for glycosylphosphatidylinositol (GPI) to have the fusion proteins anchored to the membrane as in natural conditions. These constructs have been transfected to mouse neuroblastoma cells (N2a) and clones expressing both fusions have been selected to assess PrP interactions.

For FRET analysis using the acceptor photobleaching method, the approach used to assess the degree of crosstalk between fluorophores consisted in collecting the donor's emission before and after photobleaching of the acceptor. Experimental conditions were selected to allow photobleaching of the acceptor without affecting the fluorescence of the donor. These results seem to assure optimal conditions for the subsequent assays to be performed.

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Embryonic stem cells and cardiac differentiation

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Cardiovascular diseases are a major cause of mortality in the world, but most of the current therapies aim only to prevent these diseases and to limit the pathological remodelling that occurs after an injury. New cell-based therapies aiming to correct for cardiac and vascular cells dysfunctions in cardiac tissue after injury have been tested in preclinical studies with encouraging results. Amongst the cells tested, it seems that embryonic stem cells (ESC) have the greatest capacity for cardiac cell differentiation and long-term cell survival. Induced pluripotent stem cells (iPS) with properties indistinguishable from those of ESC have now been generated by reprogramming of mouse or human somatic cells and hold a promising clinical potential for cell-based therapy. Unlike most of the adult cells, ESC and iPS are able to proliferate in culture and represent an unlimited supply of cells for therapy. However, risks of tumor formation and cellular rejection due to pluripotent nature of ESC and low efficiency of cardiac differentiation processes keep holding back their clinical application. Thus, understanding the molecular mechanisms involved in the differentiation processes of ESC and iPS cells is essential to overcome these issues. Cited2 is a transcriptional co-activator critical for normal heart development, and we are now studying its role in mouse ESC differentiation towards cardiac cell lineages.

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The role of *Ccbe1* in cardiac differentiation of mouse embryonic stem cells

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Embryonic stem cells (ESCs) represent a unique experimental model to investigate the principles of mammalian cell differentiation and development. Indeed, the expression patterns of several key cardiac genes during ESC differentiation have been shown to closely reflect their endogenous expression during cardiogenesis *in vivo*.

Collagen and calcium-binding EGF-like domain 1 (*Ccbe1*) was firstly identified using an Affymetrix GeneChip system to compare the transcriptome of chick heart precursor cells to non-cardiogenic control cells. *Ccbe1* has since been described to be involved in angiogenic sprouting in zebrafish and associated with lymphatic dysplasia and hypertrophic cardiomyopathy in humans. Related with this, EGF family molecules have also been shown to be important for cardiac development as gene targeting approaches revealed that EGF signalling is required for proper cardiac development. In mouse, *Ccbe1* is expressed at very early stages in cardiogenic mesoderm. Whole mount *in situ* hybridization and histology techniques have shown that *Ccbe 1* is expressed specifically on the cardiac progenitors regions at E7.5 (intra-embryonic mesoderm) and E7.75 (cardiac plate) and in the myocardial tissue forming the primitive heart tube at E8.5.

Recently, we have started to use mouse ESCs to assess the role of *Ccbe1* on cardiac differentiation. Overexpression and knockdown of *Ccbe1* gene will be carried out in mouse ESCs and the expression of stage-specific markers of cardiac development assessed at the protein and transcript levels. Concurrently, using the ESC technology, we are generating a null allele of this gene in the mouse in order to determine its role in cardiogenesis.

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Cited2 controls somatic and embryonic stem cell fates

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The common regulators necessary for the maintenance of somatic and embryonic stem cells (ESC) remain poorly defined. We report a requirement for the p300/CBP-binding transcriptional co-activator Cited2 in adult haematopoietic stem cell (HSC) and ESC maintenance. Conditional deletion of Cited2 in the adult mouse resulted in loss of HSCs causing multi-lineage bone marrow failure. Additional deletion of Ink4a/Arf (encoding $p16^{Ink4a}$ and $p19^{Arf}$) or Trp53 (encoding p53, a downstream target of $p19^{Arf}$) in Cited2-deficient mice rescued HSC activity indicating that Cited2 maintains adult HSCs, at least in part, via Ink4a/Arf and Trp53. Moreover, Cited2 depletion in both mouse and human ES cells resulted in loss of stem cell functions. Cited2 directly activated the expression of Nanog suggesting a novel Cited2-Nanog pathway in the maintenance of ES cell pluripotency. Taken together, our studies indicate that Cited2 is a conserved master regulator of stem cell fates and controls diverse genetic pathways to orchestrate somatic and embryonic stem cell functions.

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Chemokine receptor expression in acute T-cell leukemia

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T-cell acute lymphoblastic leukemia (T-ALL) is a neoplasia that affects 1 in 3 children diagnosed with cancer. Chemokines are molecules with chemotaxic capability and are associated in biological processes such as cell migration and differentiation. In the past years, several findings indicate that chemokine overexpression in several types of cancer induces cell survival, migration, invasion and metastization. The CCR7, CCR9, and CXCR4 chemokine receptors are important for T-cell development in the thymus, so we plan to evaluate their function in T-ALL. We also will assess the role of PSGL-1 in this disease, which is a protein that can bind the CCR7 ligands, CCL19 and CCL21. Thus, we analyzed the mRNA expression levels for these genes by semi-quantitative reverse transcription (RT)-PCR and quantitative real-time PCR in several T-ALL cell lines. All T-ALL cell lines expressed CXCR4 and PSGL-1 mRNA. CCR9 expression was detected in all but one T-ALL cell line, whereas CCR7 expression was found in 50% of T-ALL cell lines tested. Cell surface CCR7 protein expression was detected in T-ALL cell lines analyzed by flow cytometry. These results support the hypothesis that chemokine receptors may function in T-ALL development.

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Molecular therapy approach against HIV-1 Vif protein

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Viral infectivity factor (Vif) is a conserved accessory protein abundantly expressed in the cytoplasm of human immunodeficiency viruses type 1 and 2 (HIV-1 and HIV-2) infected cells, to which key role functions have been attributed during viral infectivity, namely the neutralization of innate inhibitory host defense mechanisms against retroviruses. Therefore, Vif is considered to be an important alternative therapeutic target for inhibition of HIV-1 viral infectivity. Strategies that prevent or limit expression of Vif are expected to be beneficial in the treatment of HIV-1 disease, since cellular antiviral factors may then effectively act against infection. One of these strategies is to use intracellularly expressed antibodies to neutralize Vif's actions. Such single-chain fragment variable (scFv) and single-domain antibodies were already generated against HIV-1 Vif [1], and shown to bind specifically and selectively to HIV-1 Vif *in vitro* [2].

In this work we explore the use of an anti-Vif specific intrabody (scFv4BL) and a single-domain (VHD) as potential anti-HIV-1 biotherapeutic agent in molecular therapy approach. Fluorescent microscopy and Förster Resonance Energy Transfer (FRET) were used to evaluate intracellular binding of these antibodies to HIV-1 Vif in animal cell cultures (HEK293T). The results show a predominant pattern of co-localization of both proteins in the cytoplasm of mammalian cells within a distance of about 10 nm, suggesting intracellular scFv4BL/Vif and VHD/Vif specific recognition and interaction.

Moreover, the successful of Vif recognition by specific antibodies lead us to test the effectiveness of anti-Vif antibodies to target Vif-expressing animal cell lines and to neutralize Vif's activity.

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Acknowledgement to the funding: Projects - IBB/CBME, LA, FEDER/POCI 2010, PTDC/BIO/69682/2006; Grants: SFRH/BD/36674/2007 and SFRH/BPD/30290/2006.

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The role of Shh in PSM mediolateral specification

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In vertebrate embryo development, segmentation relies on the progressive formation of early embryonic vertebrae precursor structures – somites - that periodically bud off from the rostral presomitic mesoderm (PSM). In the chick embryo, a pair of somites is formed every 90min and this periodicity is regulated by PSM cyclic expression of somitogenesis clock genes, belonging to different signalling pathways. Recently, notochord-produced *sonic hedgehog* (*shh*) has also been shown to integrate this regulatory molecular network [1]. It is known that only the medial-most PSM (M-PSM) cells possess intrinsic information for both somite formation and molecular clock gene expression, suggesting that M-PSM and lateral PSM (L-PSM) cells are differently committed to segment [2]. In the present work, the distinct specification of medial and lateral PSM was further evaluated. By performing complementary quail-chick grafts, we confirm that M-PSM cells posses an intrinsic ability to segment. Moreover, insertion of permeable and impermeable barriers to separate M- and L- PSM indicates that a diffusible signal from the M-PSM recruits and instructs L-PSM cells to form somites. We propose that the signaling molecule implicated in this phenomenon is the notochord-derived Shh. Preliminary results obtained using *in vitro* explants culture are presented and the role of Shh in medial-lateral segmentation is discussed.

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Antimalarial exposure delays *Plasmodium falciparum* intra-erythrocytic cycle and drives drug transporter genes expression

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Multi-drug resistant Plasmodium falciparum is a major obstacle to malaria control and is emerging as a complex phenomenon. Mechanisms of drug evasion based on the intracellular extrusion of the drug and/or modification of target proteins have been described. However, cellular mechanisms related with metabolic activity have also been seen in eukaryotic systems, e.g. cancer cells. Recent observations suggest that such mechanism may occur in P.falciparum.

We therefore investigated the effect of mefloquine exposure on the cell cycle of three P.falciparum clones (3D7, FCB, W2) with different drug susceptibilities, while investigating in parallel the expression of four genes coding for confirmed and putative drug transporters (pfcrt, pfmdr1, pfmrp1 and pfmrp2).

Mefloquine induced a previously not described dose and clone dependent delay in the intra-erythrocytic cycle of the parasite. Drug impact on cell cycle progression and gene expression was then merged using a non-linear regression model to determine specific drug driven expression. This revealed a mild, but significant, mefloquine driven gene induction up to 1.5 fold.

Both cell cycle delay and induced gene expression represent potentially important mechanisms for parasites to escape the effect of the antimalarial drug.

Veiga MI, Ferreira PE, Schmidt BA, Ribacke U, Björkman A, Tichopad A, Gil JP. “Antimalarial exposure delays Plasmodium falciparum intra-erythrocytic cycle and drives drug transporter genes expression.” *PLoS One*. 2010 Aug 25;5(8). pii: e12408.

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Self-assembled polymeric nanofibers for mouse embryonic stem cell culture

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Embryonic stem (ES) cell research has emerged as a vibrant area in biomedical breakthrough. This is evidently because ES cells have the ability to proliferate indefinitely in an undifferentiated state (self-renewal) and the potential to differentiate into various cell types depending on specific signals (pluripotency).

Conventionally, ES cells have been cultured on gelatin or under a monolayer of mouse embryonic fibroblasts mitotically inactivated, which differ drastically from the environment of a whole organism and consequently, cells isolated from higher organisms frequently alter their metabolism, morphology and gene expression profile. In addition, the existing complex culture systems formed from animal-derived biomaterials pose problems for replacement therapies and contain residual growth factors and undefined constituents.

The present work brings us a new artificial nanofibers structure formed from self-assembly of amphiphilic biodegradable peptide-copolymers that was designed for ES cell culture. This newly developed synthetic system allows elimination of animal-derived products and provides a complex network of nanofibers in a scale similar to the native extracellular matrices.

Undifferentiated mouse embryonic stem (mES) cells were cultured using the polymeric nanofibers in order to evaluate their potential for applications in ES cell research. It was assessed the morphology, proliferation, survival rate, self-renewal and pluripotency of mES cells. In this preliminary study, the results show that the nanofibers promoted mES cells growth and seem to retain their undifferentiated state.

Testing pluripotency and self-renewal of mES cells cultured in the nanofibers and in others already available culture systems will allow us to evaluate the potential of the new network for future applications in ES cell research and regenerative therapies.

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ENVIRONMENTAL BIOTECHNOLOGY AND CHEMISTRY

Poster Communications

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Biological fermentation of syngas

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Syngas (synthesis gas) is produced during the gasification of different materials, e.g. coal, oil and natural gas, tar sands, recalcitrant wastes, lignocellulosic biomass. Syngas is composed of mainly H_2, CO and CO_2 that can be used in a biological process for the production of fuels or usable chemicals. The objective of this work is to explore the potential of converting syngas to methane or other combustibles, such as ethanol or butanol. Mesophilic (37ºC) and thermophilic (55ºC) microcosm experiments were performed with synthetic syngas mixtures as sole carbon and energy sources. Mesophilic and thermophilic microcosms were inoculated with suspended sludge from anaerobic bioreactors. During incubations, headspace composition was analysed by GC and fatty-acids and alcohols present in the liquid by HPLC. Microbial community changes were monitored by PCR-DGGE. At mesophilic conditions, CO was not used at concentrations higher than 0.15bar. At 55°C, after successive transfers using 0.15bar, all CO was consumed and converted mainly to acetate. The thermophilic suspended sludge showed to be less inhibited by presence of CO than the mesophilic suspended sludge. Currently, series of thermophilic experiments, using a CO partial pressure of 0.3bar, is ongoing in order to adapt microbial communities to higher CO concentrations. A thermophilic enriched culture, consisting of two microorganisms and able to convert syngas was obtained. Identification of these microorganisms is being conducted using cloning and sequencing techniques. We expect to isolate these bacteria in order to characterize their physiology regarding CO conversion and to explore their potential for biotechnological applications.

The financial support from Fundação para a Ciência e Tecnologia (FCT) trough the PhD grant SFRH/BD/48965/2008 given to Joana I. Alves is gratefully acknowledged.

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Applications of quantitative image analysis in wastewater treatment

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Quantitative image analysis (QIA) techniques gained an undeniable role in several fields of research during the last decade. In the field of wastewater treatment (WWT) processes, several computer applications were developed for the monitoring of microbial entities either individual cells, or several types of aggregates. New descriptors were defined that are more reliable, objective, and useful than the subjective and time-consuming parameters used classically to monitor the WWT biological processes. Examples of application include the objective prediction of filamentous bulking, known as one of the most problematical phenomenon occurring in activated sludge technology. It also demonstrated to be useful to classify protozoa and metazoa populations. In high rate anaerobic processes, based on granular sludge, it was possible to detect aggregation time and fragmentation phenomena during critical events, such as toxics and organic overloads. Currently, the major efforts on the development of QIA techniques in WWT technology are focused in its application coupled with coloured samples, obtained after staining and fluorescent techniques. Also, the use of quantitative morphological parameters in process control is being investigated. In fact, employ multivariate statistical analysis to data gathered by QIA during transient states of an anaerobic reactor determined a latent variable that encompasses a weighted sum of performance, physiological and morphological information. This new variable was highly sensitive to reactor efficiency deterioration, enclosing remarkable variation in the first hours of the disturbances. The high loadings raised by morphological parameters revealed that they should be considered to monitor and control load disturbances and toxic events in high rate anaerobic digesters.

Intensified bioprocess for the anaerobic conversion of syngas to biofuels

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Syngas, composed mainly of CO, H_2 and CO_2 can be produced from several sources, including coal, oil and natural gas, tar sands, recalcitrant wastes and biomass. Syngas can be a potential feedstock for the sustainable production of biofuels and bulk chemicals. The selective biological conversion of syngas is a possible alternative to the chemical route. Nevertheless the biological route remains rather unexplored within the bioprocess engineering community. Some anaerobic micro-organisms have the ability to use CO, H_2 and CO_2 and produce renewable biofuels such as ethanol, butanol, and methane. Recently, the microbiology of syngas fermentation has been extensively reviewed (e.g. [1]). As in the stage of work planning, this work introduces the main issues in the topic of syngas fermentation to biofuels. The experimental work to be performed aims to develop a new anaerobic bioprocess for the conversion of syngas to biofuels, with principal interest in ethanol, butanol, and CH_4. An oscillatory flow reactor (OFR), presenting efficient gas-liquid mass transfer rates, will be explored carrying out proof-of-concept experiments using pure and defined mixed anaerobic cultures. Previous results from oxygen mass transfer studies [2] performed in an OFR, have shown effective improvement in gas-liquid mass transfer, when compared with conventional gas-liquid contacting technologies. In a later stage of this work, an energy based metabolic model will be developed to predict products formation according to specific environmental conditions.

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Bioremoval of hexavalent chromium by *A. viscosus* supported on Y and ZSM5 zeolites

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The removal of Cr(VI) by a system that combines biosorption by a bacterium with the ion exchange properties of a zeolite was evaluated. The aim of this work was the assessment of the effect of sodium content in Y and ZSM5 zeolites, on the removal of chromium. The zeolites NaY, HY and ZSM5 were used as the starting materials. The modified zeolites H(Na)Y and H(Na)ZSM5 were obtained by an ion exchange treatment with $NaNO_3$ solution. Batch biosorption assays were performed, using an initial Cr(VI) concentration of 100 mg/L and a biomass concentration of 5 g/L. During the biosorption process, *Arthrobacter viscosus* bacterium supported on zeolite performed the reduction of Cr(VI) to Cr(III), which was retained in the zeolite by ion exchange [1]. After the biosorption assays, the zeolite loaded with chromium were analysed by bulk chemical analysis. The results revealed that the initial sodium content in the zeolite had an effect on the removal of chromium by the zeolites. For the Y zeolite it was achieved the highest chromium content for the starting zeolite that had the lowest sodium content, HY. The same tendency was observed for the ZSM5 zeolite, but in this case the increase of sodium in the structure led to a significant drop of chromium percentage in the zeolite. In fact, the ion exchange of a small cation, such as H^+, is easier in comparison to Na^+, and the reduction of Cr(VI) by the bacterium is enhanced with a higher concentration of protons.

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Assessment of the aerobic granulation process through quantitative image analysis

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The present work aims to monitor the aerobic granulation process through quantitative image analysis. The image processing and analysis programme developed by Amaral (2003) [1] for bright field microscopy was used to obtain the aggregates content and the aggregates morphology.

Aerobic granulation of activated sludge was achieved in a sequencing batch airlift reactor (SBAR) fed with acetate as sole carbon source. The SBAR was operated in 4 hour cycles, with 2 minutes settling time that promoted the selection of biomass with a minimum settling velocity of 11 m/h. Compact aggregates with granular characteristics, i.e. aggregates with equivalent diameter higher than 0.25 mm, were identified after 4 days of operation. The system was monitored through influent and effluent characterisation in terms of solids and substrate concentration, and biomass characterisation in terms of concentration, settling ability, density, and aggregates morphology. Aggregates size was divided in four classes: (i) larger granules with equivalent diameter above 1 mm, (ii) smaller granules with equivalent diameter between 0.25 and 1 mm, (iii) intermediate size aggregates with equivalent diameter between 0.025 and 0.25 mm, and (iv) smaller aggregates with equivalent diameter below 0.025 mm. The obtained results allowed to successfully monitor the aerobic granulation, essentially the aggregates size distribution provided valuable information on aerobic granulation progress.

The obtained results showed that quantitative image analysis is a powerful tool to monitor the aerobic granulation process.

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Phosphorus Mobility in Lake Sediments

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For a long time that phosphorus release in sediments has been almost exclusivelyrelated with the absence of oxygen in the hypolimnium. This paradigm was firstly statedwith the pioneer work of Einsele (in 1936) and Mortimer (in 1941), demonstrating therelation between the reduction of Fe (III) and phosphorus release, in anoxic sediments.

Although the theoretical statements matched the practical findings, these could not begeneralized since several field observations and laboratory experiments lead to otherconclusions. In fact, according to this paradigm, restoration measures where appliedusing hypolimnetic aeration, but with few or no results in eutrophication control. Thisacquired experience as lead to a need of deeper understanding of factors thatinfluence the phosphorus mobility, leaving the old paradigm applicable only in specialcases. Enumerating some of the factors, studies have shown that phosphorus releaseis largely influenced by the phosphorus retention capacity of the lake due to thesediments' geochemical characteristics. The presence of metallic oxides andhydroxides (specifically of aluminium and iron) in the sediment structure enhanceretention, even in anoxic conditions. Phosphorus can also be released by microbialmediation in processes like organic matter mineralization, iron and sulphate reduction.

Bacteria can also impel a pH and redox variation that consequently leads togeochemical change liberating phosphorus. In the other hand, some bacteria have thecapability of storing phosphorus. A review of these processes drives to a betterunderstanding of factors that controls phosphorus mobility in natural waters.

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Prokaryotic and eukaryotic populations in activated-sludge

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Protozoa play a direct role by reducing through grazing the amount of freely-suspended and loosely-attached bacterial cells. Also, filamentous bacteria, although endangering the performance of wastewater treatment plants (WWTP), should be considered as normal components of the activated-sludge microbial community. Correlations between plant performance and the abundance of certain species have been studied, being the Sludge Biotic Index the best known method to assess the activated-sludge plant performance through the analysis of protozoa and small metazoan communities. However, few studies have established reliable relationships between the prokaryotic and eukaryotic populations.

The present work presents data on the prevalence, abundance and distribution of protozoa, small metazoa and filamentous bacteria on 37 activated-sludge Portuguese WWTP operating under different environmental conditions, during one year, including data on the correlations between the prokaryotic and eukaryotic components.

The most frequent protozoa were the crawling (CC) and the attached sessile (ASC) ciliates, being *Aspidisca cicada*, *Epistylis* spp. and *Microthorax* sp. the most abundant. The most frequent filamentous bacteria were Types 0041/0675, 0092, 1851, Nocardioforms, *Microthrix parvicella, Nostocoida limicola* II *and Haliscomenobacter hydrossis*; only the former four were found dominant in all samples. Correlations were found to be significantly positive ($p<0,05$) between *Nostocoida limicola* II and Type 0092 and negative between *Thiothrix* II and *Microthrix parvicella*. Correlations between filamentous bacteria and protozoa were significantly positive ($p<0,05$) for freely swimming ciliates (FSC) and Type 0092 and for flagellates (F)/*Thiothrix* II. Negative correlations were found for FSC and *Microthrix parvicella*, F and Nocardioforms.

Green algae as source of a polysaccharide – ulvan – suitable for biomedical applications

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The sustainable exploitation and valorisation of natural marine resources represents a highly interesting platform for the development of novel biomaterials, with both economic and environmental benefits. Marine organisms are composed by different molecular components with a vast range of properties and characteristics that justify their study in potential applications within the biomedical field. In this perspective, an increasing number of different molecules are being isolated from aquatic organisms; among these polysaccharides play a decisive role. The green alga *Ulva* contains three main polysaccharidic components: unusual amorphous cellulose, water soluble anionic polysaccharides containing sulphate groups and starch [1]. Ulvan is a water soluble sulphated polysaccharide present at the cell wall of green algae that is aimed, among other functions, to ensure that water in the extracellular environment is kept during low tide periods, while at shore [2]. Taking into account the intrinsic characteristics of this polysaccharide and the high valorisation potential of this unexploited marine biomass, it is of great scientific and technological interest to investigate the application of ulvan as a potential biomaterial in several biomedical applications, including tissue engineering. A novel extraction methodology was defined in order to extract ulvan from the green alga *Ulva lactuca*. The obtained polysaccharide was further characterized in order to unravel its physicochemical characteristics and its biological performance was assessed by means of *in vitro* cytotoxicity. Furthermore, the processability of ulvan into tissue engineering scaffolds is currently being studied.

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Adaptation of bacteria to extreme conditions: can we create extremophiles?

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Extremophiles have been isolated from samples collected under conditions of extreme temperature (-2º to 15ºC or from 60º to 100ºC), ionic strength (e.g. 2 to 5 M NaCl) or pH (below 4 or above 9). The interest in extremophiles increased significantly when it was found that their survival depended on novel enzymes and biochemical pathways which could be used to human benefit e.g. to produce food additives, detergents and pharmaceuticals [1]. The particular abilities of these microorganisms are also the result of thermostable membrane proteins, high turnover rates of various enzymes and especially unique membrane lipid composition.

Adaptation of the fatty acid composition of the cellular membrane is a known mechanism of cellular adaptation to temperature, pH, salt concentration and organic solvents [2]. Among the adaptive mechanisms described, those leading to modifications of the membrane to keep the same level of fluidity have been reported most intensively.

Rhodococcus erythropolis cells were adapted to low and high temperatures (below 15ºC and above 32ºC), salt concentrations above 5%, to extreme pH values (below 4 and above 9) and to copper concentrations higher than 0.5%. The fatty acid composition of the cellular membrane, the viability and morphology of cells and the membrane potential were analysed to clarify the adaptation mechanisms involved.

The cells were able to grow in situations quite different from the normal optimum conditions and still to metabolise alcohols and hydrocarbons. This indicates that some bacteria can be adapted to extreme conditions.

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Production of energy with *Rhodococcus* cells

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Renewable fuels from suitable, regenerative and ecologically friendly processes to replace petroleum-derived combustibles are required. Biodiesel has been considered a good candidate to substitute diesel obtained from crude oil, but the use of food crops as the starting material has raised several ethical and economic issues.

Algae, yeast and bacteria are known to produce triacylglycerols (TAGs), the most relevant lipids for biodiesel production. In bacteria, accumulation of TAGs has only been described in the genera *Mycobacterium*, *Streptomyces*, *Acinetobacter*, *Nocardia* and *Rhodococcus* [1]. Rhodococcus opacus PD630, grown on gluconate medium, is capable of accumulating up to 76% of the cell dry weight in TAGs.

In the present study, the production of TAGs in *R. erythropolis* was assessed and the conditions allowing the highest TAG yield were determined. Furthermore, the cells were able to carry out the biocracking of long chain alkanes, producing short chain alkanes that could be used as biofuel. *R. erythropolis* DCL14 cells were also found able to adapt the surface net charge according to the carbon source used [2]. This allowed the micro-generation of electricity between reactors containing cells grown on different carbon sources.

The results obtained so far indicate that *Rhodococcus* cells could be used as an alternative energy source, although significant amount of work is still required for large scale energy production.

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Ecological impact of wastewater in the diversity and structure of macroinvertebrate communities in the Zêzere River

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Ecological impact of waste water treatment plants (WWTPs) discharges and surrounding industrial activities on river Zezere is being assessed through the analysis of the distribution and abundance of benthic macroinvertebrate and microhabitat dynamics along the first 98 Km (242 km total length) of the mountain stream in Serra da Estrela, located in the NW region of the Tagus river basin. Sample collections were carried out during the spring and summer of 2007, fall of 2008, winter and spring of 2009 and 2010, in nine ecotones divided into four aquatic habitats (bedrocks, gravel substrates, submerged roots and vegetal margins). Significant changes in macroinvertebrate diversity were observed in river downstream and macroinvertebrate communities changes correlated with water physical and chemical parameters variation. Spatial distribution showed that the Batidae (Ephemeroptera); Perlodidae (Plecoptera); Limnephilidae, Psychomyiidae (Trichoptera) taxa are steady populations concerning the number of individuals, habitat and time of sampling collection. Six families of the Trichoptera taxa were found and Polycentropodidae, Psychomyiidae were identified as the most sensitive species to pollution water. Diptera order community structure and abundance, especially of the Chironomidae, Simuliidae, Ceratopogonidae, Tipulidae, Limoniidae and Empididae families, revealed a poor water quality denoting the negative impact of WWTPs, in the four neighbouring ecotones. The highest values for biodiversity were found in summer in the bedrocks and in the gravel substrates. Agriculture, aquaculture, wastewater treatment plants (WWTP´s) and mining activities caused significant changes in the macroinvertebrate abundance and community structure along the river basin and thus demonstrate the existence of downstream ecological pollution gradient.

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The reproductive success of the invasive clam *Mya arenaria* (Bivalvia) in the Tagus estuary

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The invasive clam *Mya arenaria* has reached a more advanced stage in the process of invasion in the Tagus estuary [1], as was revealed by a three month field experiment comprising tidal level (upper, middle, lower) and treatment (excavated and non excavated plots) as categorical experimental factors. The observation of the smallest recruited juveniles of *Mya arenaria* (2 mm) throughout the period of study, lead us to the conclusion that the clam is capable of reproducing in the new habitat. Juveniles of both *Mya arenaria* and the bivalve *Scrobicularia plana* were found to avoid excavated experimental plots, showing a significantly higher abundance in control plots. These data, strongly suggest that the recruitedbivalves actively avoided unsuitable substrata. Juvenile specimens of *Mya arenaria* were more abundant in the mid intertidal region. However, juvenile specimens of *Scrobicularia plana* were mainly distributed in the upper intertidal level, suggesting a different settlement behaviour than that observed for the juveniles of the invasive clam. Despite of the divergent distribution between the juveniles of both species in the study site, a likely interaction between these two species is considered and discussed.

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Effect of a respiratory inhibitor on the bioconversion of a xenobiotic by activated sludge

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Xenobiotic bioconversions often involve oxidation-reduction reactions and one representative example is the reductive decolorization of azo dyes. The bioreduction of an azo dye by a mixed microbial culture (activated sludge) was chosen as a model system in the present study. The effect of a respiratory inhibitor, sodium azide, on this bioconversion, when carried out by different microbial inocula, was investigated. The dye used was Acid Red 14, with two cultures, one from an aerobic, laboratory-scale sequential batch reactor, fed with glucose, and another from the aeration tank of a municipal wastewater treatment plant. The bioconversions were carried out in duplicate, in closed recipients with reduced headspace to induce anaerobic conditions, with glucose as electron donor. Dye bioreduction was followed by UV/visible spectrometry, with 1-5% of observed dye removal being attributed to adsorption onto the biomass. Bioconversion yield values above 90% were observed with both mixed cultures after 48 hours, with or without added glucose, but bioconversion inhibition by azide showed different patterns, depending on biomass preconditioning, i.e., freshly harvested or after aerobic incubation (24 to 96h) in the absence of carbon source (starved). Azide inhibition (60 and 200 mM) of dye bioconversion was observed with the fresh cultures from both sources. After starvation, both cultures showed a reduction in the azide inhibition effect. However, in the absence of glucose this alleviation of azide inhibition was not observed. These results point to the existence of an alternative, dye bioconversion mechanism triggered in the starved biomass, not inhibited by azide.

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Phytotoxicity tests as an indicator for phytoremediation performance

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The plants tolerance to different pollutants levels represents an important issue to be considered prior to any soil phytoremediation implementation. Phytotoxicity test based on the germination index is a simple, rapid and cost-effective technique to monitor the plants resistance to contaminants.

To evaluate soil/plant toxicity of an old constructed wetland used to treat recalcitrant compounds different soil samples were collected along beds and at different depths. The soil was incubated, at 25ºC, for four days, with *Lepidium sativum* seeds [2]. The number of germinated seeds and the plant development (weight and length) are determined. Different amounts of amino and nitro benzene compounds were also mixed up with the soil samples in order to set its non-toxicity limits. Seeds germination index was almost 100% in all cases and plants grew at high rate using organic compounds, as carbon source. In some cases the plant growth and the number of seeds germinated was twice higher than reference.

So far toxicity levels were found within the range of 0.02 to 0.08 mmol/kg_{soil} quite above the reported values.

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Phragmites sp. peroxidases role in azo dyes degradation

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Encouraging results obtained on the degradation of the mono azo dye Acid Orange 7 (AO7), using a vertical flow CW, indicates that mineralization of this compound was possible [1]. In order to clarify the role of *Phragmites sp.* in AO7 detoxification process, activities of several antioxidative enzymes involved in plant protection against oxidative stress were assayed and found to be enhanced [2].

Peroxidases (POD) enzymes belong to a family of isozymes found in all plants. POD are heme proteins able to extract one or two electrons from an organic substrate using H_2O_2 as electron acceptor. In the present work, a crude extract obtained from *Phragmites* young green leaves was used to evaluate their POD ability to use several azo non-reactive dyes as donor substrates. In fact, POD were able to degrade mono azo dyes AO6, AO7, AO20, AO52, AR66, AY17, AY23, AY36, DR81 and FY3 but had no effect on di-azo dyes AB113 and AB120. Moreover no correlation between dye stereochemistry, molecular weight and octanol-water partition coefficient in the decolourization rate by plant leaf extract was found.

Phragmites sp. POD activity characterization denotes a high specificity towards substrate leading to variations from 1 to 10^10 U/mg of protein which is intriguing and denotes some “plant memory” when dealing with pollutants that have been already in contact with the plant. Five POD isoenzymes were already detected by SDS-PAGE electrophoresis.

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Novel method for gas-liquid mass transfer coefficient determination in gas-liquid-liquid dispersions

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A new, simple and cheap method for gas-liquid mass transfer coefficient determination in gas-liquid-liquid dispersions was devised and implemented. The rate of solute transferred is obtained by measuring the inlet/outlet concentration of an organic volatile being transferred to/from the liquid phases and performing a mass balance. Knowing the organic liquid composition and the gas composition, the driving force can then be calculated, allowing determination of the volumetric mass transfer coefficient.

The system under study was an aerated stirred tank containing a heptane/dodecane solution dispersed in water. The solute transferred (absorbed or desorbed) was heptane. Measurement of heptane in the gas phase was carried out using a cheap photo ionization detector, normally used for VOC monitoring, calibrated for heptane as the single solute.

The method was tested by comparing mass transfer coefficients thus measured with oxygen mass transfer coefficients obtained by the pressure step dynamic method, and accounting for the difference in diffusivity of the two solutes. Agreement between the two methods is reasonable, errors being attributable to either method.

The proposed method can however only be applied when the organic phase spreading coefficient is negative, i.e., when the organic liquid phase does not tend to spread onto the gas-liquid interface.

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Studies on the development of novel heterogeneous catalysts for transesterification of triglycerides in biodiesel

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The transesterification reaction of triglycerides is of considerable interest in the production of biodiesel, which is currently manufactured by transesterification of triglycerides with methanol, using liquid NaOH or KOH. Catalysts as such are somewhat corrosive to the equipment, but this fact could be overcomed with the construction of the reaction vessels in stainless steel, with increased costs. Another drawback is that the liquid based catalyst has to be neutralized after the end of the reaction, typically using HCl, thus producing salt streams. Moreover, due to the presence of free fatty acids it reacts to form soaps as unwanted by-products, hence requiring more expensive separation processes. In this particular process, the biodiesel composition greatly depends on the content of raw fatty material, namely on the types of fatty acid groups forming the triglyceride. Another possibility to achieve esterification is to use H_2SO_4 as catalyst in a batch-wise process. The problem here is that the batch operation mode is not suitable for very large-scale production and, again, involves costly neutralization and separation of the homogeneous catalyst.

Therefore, there is currently a drive towards the development of industrial processes for biodiesel production using solid catalysts [1-2]. The key benefit of using solid acid or basic catalysts is that no polluting by-products are formed and the catalysts do not have to be removed since they do not mix with the biodiesel product. In addition to lower separation costs, less maintenance is needed as these catalysts are not corrosive.

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Study on the activity of new amine based absorbents for CO_2 absorption from flue gases

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The capture of CO_2 from flue gases can be done by chemical absorption with aqueous amine solutions like MEA (monoethanolamine). Although this is a proven process, there are still operational problems to overcome [1], namely those resulting from solvent degradation, precipitation, corrosion and foaming. Also, the absorption capacity of the most current amines must be improved in order to obtain a more profitable operation. It has been shown previously that relationships exist between the amine structure and the activity/capacity for CO_2 absorption. The introduction of amine substituents at the α-carbon creates a carbamate instability, which causes the hydrolysis to go faster, thus increasing the amount of bicarbonate, allowing for higher CO_2 loadings. In order to obtain a better understanding of the structure-activity relationship, laboratory studies have been made comprising solvent screening experiments investigating the effect of variables such as chain length, increase in number of functional groups, side chain at α-carbon position, alkyl group position in cyclic amine and side effect of cyclic amine with different functional groups. The description of these effects, in a quantitative way, on the initial rate of absorption for CO_2, as well as the capacity of various solvents for CO_2 absorption is also supported by simulation using ASPEN/HYSYS and it is believed to benefit on the design of more efficient absorption systems for CO_2 capture from flue gases.

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AGRO-FOOD BIOTECHNOLOGY

Poster Communications

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Evaluation of filamentous fungi for the treatment of olive mill waste-waters

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In Portugal, olive mill wastewater (OMW) production is estimated at $100x10^3$-$350x10^3$ m^3/year and constitutes an important seasonal pollution problem to be solved by the local olive oil industry. OMW is a dark liquid residue with high organic content composed mainly by sugars, tannins, polyphenols, polyalcohols, organic acids, proteins, pectins and lipids. Different treatments and disposal alternatives can be found in the literature in order to provide solutions for the OMW problem. OMW biological treatments with its simultaneous valorisation through the co-production of added-value products are one of the main approaches possible. The aim of this work is to evaluate the capability of several filamentous fungi to reduce the polluting characteristics of OMW and to produce extracellular enzymes. Under this study, strains of *Trametes versicolor*, *Aspergillus ibericus*, *Aspergillus niger* and *Penicillium expansum* were tested. Agitated batch fermentations were carried out at 27 ºC for seven days using culture media prepared with different concentration (10, 50 and 100 %) of centrifuged OMW. Daily changes on the concentration of reducing sugars, protein, phenolic and aromatic compounds, COD, colour and pH were determined. Additionally, produced lipase, lignin and manganese peroxidase and laccase were followed. When 10 % of OMW was used, *T. versicolor* and *A. ibericus* were found to be the most effective strains, reducing OMW colour (30 and 55 %, respectively), aromatic compounds (16 and 39 %, respectively), phenolic compounds (55 and 37 %, respectively) and COD (55 and 39 %, respectively). In those same conditions, the maximum lipase activity was 108 U/L, 291 U/L, 77 U/L and 47 U/L for *T. versicolor*, *A. ibericus*, *A. niger* and *P. expansum*, respectively. For the oxidative enzymes, activity was only detected in fermentations conducted with *T. versicolor* - maximum recorded activity was 12 U/L and 83 U/L for laccase and lignin peroxidise, respectively. When 100 % of OMW was used, *A. ibericus* reduced OMW colour and COD by 97 % and 45 %, respectively, but no reductions of aromatic and phenolic compounds were observed. In those conditions lipase produced by *A. ibericus* reached 2927 U/L.

Luís Abrunhosa and Cristiana Gonçalves want to acknowledge the support from Fundação para a Ciência e Tecnologia (FCT) from Portugal (grant SFRH/BPD/43922/2008 and SFRH/BD/27915/2006 respectively). The authors also acknowledge FCT for the financial support through the project PTDC/AMB/ 69379/2006.

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Micoteca da Universidade do Minho (MUM): a Portuguese Culture Collection

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Micoteca da Universidade do Minho - MUM is a mycological culture collection that exists since 1996 and is hosted by the Biological Engineering Research Centre (www.deb.uminho.pt), a centre of excellence integrated in the Institute for Biotechnology and Bioengineering (IBB - www.ibb.pt). The mission of MUM is to provide the highest quality services to its customers, collecting, maintaining and supplying fungal strains and their associated information for teaching and research in biotechnology and life sciences, and to be a centre of knowledge, information and training in mycology, operating at a global level and under national and international regulations [1,2].

MUM is a member of the European Culture Collections Organization (ECCO) and the World Federation for Culture Collections (WFCC) and is also registered with the World Data Center of Microorganisms (WDCM) and with the network Biological Collection Access Service for Europe (BioCase). MUM was involved in the Working Group of Organisation for Economic Co-operation and Development (OECD) for Biological Resource Centres, is involved in the Global Biodiversity Information Facility (GBIF), and is a partner of Global Biological Resources Centre Network (GBRCN) and of the project European Consortium Microbial Resources Centres (EMbaRC).

MUM intends to maintain its international status and to act in network with other collections, valuing costumer's needs and supplying high quality products and services.

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Nanostructured films developed through layer-by-layer assembly of κ-carrageenan-chitosan

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Nanotechnology holds a great potential to generate very innovative solutions and to provide food technologists with instruments to meet the ever-growing consumer demands in very diverse aspects related with the foods they eat: safety, quality, health-promotion and novelty. Layer-by-layer assembly, which is performed by the simple alternating immersion of substrates into aqueous solutions of oppositely charged polyelectrolytes, can be applied to produce multilayers of nanometer thickness on various surfaces. Multilayered coatings can be specially engineered to incorporate and allow the controlled release of bioactive compounds and can be used to coat food systems such as fresh-cut fruits and vegetables.

The aim of the present work was to develop a multilayer coating through layer-by-layer assembly technique using two oppositely charged polysaccharides, κ-carrageenan (zeta potential of -57 mV) and chitosan (zeta potencial=+46 mV), onto aminolyzed/charged polyethylene terephthalate (PET) and to characterize the film in terms of its permeabilities and surface properties. The □-carrageenan/chitosan system was chosen for this study due to their water barrier and antimicrobial properties, respectively.

The adsorption of the polyelectrolytes on PET surfaces was monitored by UV-VIS spectroscopy, quartz crystal microbalance and contact angle measurements and analysed by scanning electron microscopy (SEM). The nanolaminates, composed by three κ-carrageenan and two chitosan layers, has been successfully assembled on PET substrate, as confirmed by the increase of absorbance and the decrease of frequency after each polyelectrolyte deposition, by changes in the contact angle and SEM (nanolayers total thickness of 171.1 nm). The κ-carrageenan/chitosan multilayers exhibits good gas barrier properties and offer great potential to be used to coat food systems.

These results will contribute to the establishment of an approach to produce edible multilayers with improved characteristics to coat e.g. fresh and minimally processed fruits, aiming at a higher benefit for the product/consumer.

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Supercritical fluid extraction of *Myrtus communis* L.: comparison of antioxidant activity in extracts obtained by SFE vs solvent extraction

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This paper reports results of a study on a shrub typical of the Portuguese flora, *Myrtus communis* L., commonly known as myrtle. This plant is described in the literature as an antioxidant (AO) source for nutraceutical purposes. We are interested in assessing its antioxidant capacity (AOC) and to that end we are using the Supercritical Fluid Extraction (SFE) technique to obtain the plant extracts to study the performance of this technique against the more conventional extraction techniques, namely hydrodistillation followed by liquid-liquid extraction (LLE) and solid-liquid extraction (SLE). Supercritical extraction studies were performed using carbon dioxide as the supercritical fluid at different experimental conditions. Cumulative extraction curves for different temperatures, 308 and 321, 333 K, and pressures 10, 15 and 30 MPa and at constant carbon dioxide flow of about $7X10^{-5}$ kg s^{-1} are presented.

The AO activity was determined by three different methods: Folin-Ciocalteu, TEAC (Trolox equivalent antioxidant capacity) and ORAC (oxygen radical absorbance capacity). They were chosen among the many methods available because overall they give a fairly accurate account of the AOC of a plant extract [1].

The results for the extracts obtained by SFE were compared with those obtained by conventional extraction showing that SFE is effective to extract AO components from *Myrtus communis* L., and the use of ethanol as co-solvent, at the studied conditions, increases the values of TEAC and ORAC of about four times, when compared to values obtained when conventional extraction is used.

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Are *Plantago algarbiensis* and *P. almogravensis* aluminium hyperaccumulators? Biochemical and physiological response

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Plantago algarbiensis and *P. almogravensis* are wild species endemic from the Western-centre of the Algarve region and the Portuguese Southwest coast, respectively. The aim of this work was to evaluate the aluminium (Al) accumulation capacity and the biochemical and physiological effects (level of lipid peroxidation, the proline and carbohydrate contents, the antioxidant enzyme activities and chlorophyll a fluorescence) of Al in micropropagated shoots of these *Plantago* species. Results demonstrated that shoots of both species accumulate amounts of Al much above 1000 µg g-1 dry weight without visible symptoms of toxicity and no effect on photochemical processes. However, for the biochemical parameters analyzed some differences were detected between both species in response to low pH and/or Al. Al induced the increase of proline and carbohydrate contents, and antioxidant enzyme activities in *P. almogravensis*, while no changes in lipid peroxidation levels were detected. On the other hand, Al induced an increase of the level of lipid peroxidation and enhanced proline levels and antioxidant enzymes activities in *P. algarbiensis*, while the carbohydrate content remained unchanged. Based on these results it can be concluded that both *Plantago* species behave as Al hyperaccumulators, but *P. almogravensis* seems to have an efficient internal mechanism of tolerance that minimizes accumulation of lipid peroxides through a higher proline and carbohydrate content and antioxidant enzyme activities.

Acknowledgements:
N. Martins, S. Gonçalves and ML Osório acknowledge grants from the Science and Technology Foundation (FCT, SFRH/BD/48379/2008, SFRH/BPD/31534/2006 and SFRH/BPD/35410/2007, respectively). This work was supported by the FCT project PTDC/AGR-AAM/102664/2008.

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Effects of acetic acid, ethanol, and SO_2 on the removal of volatile acidity from acidic wines by two *Saccharomyces cerevisiae* commercial strains

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Herein, we report the influence of different combinations of initial concentration of acetic acid and ethanol on the removal of acetic acid from acidic wines by two commercial *Saccharomyces cerevisiae* strains S26 and S29. Both strains reduced the volatile acidity of an acidic wine by 78% and 48%, respectively. Acetic acid removal by strains S26 and S29 was associated with a decrease in ethanol concentration of 0.7 and 1.2% (v/v), respectively. Strain S26 revealed better removal efficiency due to its higher tolerance to stress factors imposed by acidic wines. Sulfur dioxide (SO_2) inhibits the ability of both strains to reduce the volatile acidity of the acidic wine used under our experimental conditions. Therefore, deacidification should be carried out either in wines stabilized by filtration or in wines with SO_2 concentrations up to 70 mg l^{-1}. Deacidification of wines with the better performing strain S26 was associated with changes in the concentration of volatile compounds. The most pronounced increase was observed for isoamyl acetate (banana) and ethyl hexanoate (apple, pineapple), with an 18- and 25-fold increment, respectively, to values above the detection threshold. The acetaldehyde concentration of the deacidified wine was 2.3 times higher, and may have a detrimental effect on the wine aroma. Moreover, deacidification led to increased fatty acids concentration, but still within the range of values described for spontaneous fermentations, and with apparently no negative impact on the organoleptical properties.

Nitrogen limitation triggers ROS production and cell cycle arrest during alcoholic fermentation in *Saccharomyces cerevisiae*

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The nitrogen limitation has strong effects on yeast physiology and metabolism, being of utmost importance to elucidate the boundary levels of nitrogen in fermentation media that have minimum effect on yeast alcoholic fermentation. On the other hand, uncovering the underlying effects nitrogen limitation can be translated into the prediction of fermentation problems during alcoholic beverage production, particularly wine making.

In the present work, reactive oxygen species (ROS), plasma membrane integrity and cell cycle were evaluated as stress biomarkers in cells of *Saccharomyces cerevisiae,* during alcoholic fermentation in nitrogen-limiting media. The results indicated that nitrogen-limitation leads to an increase of ROS production, where superoxide anion seems not to play a relevant contribution. Together with these effects an increase of loss of plasma membrane integrity and a persistent arrest of cells in G0/G1 cell cycle phases were observed. Moreover, under these conditions it appears that autophagy, evaluated by *ATG8* expression, is being induced, suggesting that it might be essential to allow cell-survival. Conversely, nitrogen feed permitted cells to re-enter cell cycle by abolishing oxidative stress and decreased autophagy. Altogether the results provide new insights on the understanding of wine fermentations under nitrogen-limiting conditions.

This work was carried out in the framework of project the PTDC/AGR-ALI/71460/2006 and PTDC/AGR-ALI/68284/2006.

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The role of nitrogen supply for modulating sulfide production by yeasts during alcoholic fermentation

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The production of H_2S by *Saccharomyces cerevisiae* during fermentation is of major concern because it imparts an unpleasant rotting odor to the wine even when present at very low concentration. H_2S is a metabolic intermediate of the tightly regulated sulfate reduction sequence (SRS), which represents part of the biosynthesis of sulfur-containing amino acids, in a low nitrogen grape-juice. The relationship between nitrogen deficiency and H_2S formation is thought to reflect the combined activation of the SRS and the depletion of intracellular nitrogenous precursors of sulfur amino acids, which would normally act to incorporate H_2S. An ineffective incorporation of H_2S can therefore lead to the formation, and ultimately the release of H_2S from the cell. Work is under way to compare yeast strains which differ markedly from one another in respect to liberation of H_2S growing under different conditions of nitrogen availability and their relationship with the expression levels of genes involved in SRS pathway. The analysis was performed at two different sampling time points. The expression patterns of the genes analyzed by real time PCR revealed that *MET3* encoding ATP sulfurylase, which catalyzes the primary step of intracellular sulfate activation, was the most highly expressed of the genes studied in strain PYCC4072, and *SSU1* in strain UCD522. *MET10,* subunit alpha of assimilatory sulfite reductase, and *SSU1,* a plasma membrane sulfite pump, in both strains, but *MET16* (3'-phosphoadenylsulfate reductase) only in PYCC4072, were consistently up-regulated when H_2S production was inhibited.

This work was carried out in the framework of project the PTDC/AGR-ALI/71460/2006

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Estimating long term stability of polysaccharide nanoparticles for intestinal Peyer's patches pathway

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Effective delivery of monoclonal antibodies (mAb) is limited by developing carriers that enhance bioavailability and ensure stability in specific biological environments. Being less user-friendly and with infection risk, parenteral administration is usually avoided and whenever possible replaced by oral delivery. However, this usually results in low bioavailability due to gastrointestinal degradation and low permeability. Polysaccharide nanomedicines have been reported to successfully deliver mAb enabling protection from degradation, promoting gut permeation and systemic uptake. With sizes up to few hundred nanometers, nanomedicines display increased surface-to-volume ratio and surface functionality, offering high potential to target a number of drugs for e.g. colitis, Crohn disease, in particular because they are easily uptaken by intestinal Peyer's patches. Ionic interaction of several polysaccharides with a counter-ion has been used to produce mAb-loaded nanomedicines. PCS, ZP and DSC analyses were undertaken for physicochemical characterization, following determination of encapsulation parameters. Selection of optimal formulations was based on a two-level full factorial design study. Four variables were taken at two levels, i.e. low (-1) and high (+1). Sixteen batches were produced following determination of loading capacity, encapsulation efficiency, mean particle size and polydispersity index (PdI) and polynomial equations were generated. Each response Yi and predictor of response was first converted to an individual function (di) varying from 0 to 1, where di=0 stands for undesirable response and di=1 for desirable/ideal response. Mathematical modelling seems promising to select appropriate materials when studying variables. In particular, mean particle size and PdI may be well controlled by statistical analysis creating a model to assess effect of individual variables and their interactions.

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Optimization of honey-must preparation and alcoholic fermentation by *Saccharomyces cerevisiae* for mead production

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Mead fermentation is a time-consuming process, often taking several months to complete. Despite of the use of starter cultures several problems still persist such as lack of uniformity of the final products, slow or premature fermentation arrest and the production of off-flavours by yeast. Thus the aim of this study was to optimize mead production through the use of an appropriate honey-must formulation to improve yeast performance alcoholic fermentation and thereby obtain a high quality product. Honey-must was centrifuged to reduce insoluble solids, pasteurized at 65ºC for 10 min., and then subjected to different conditions: nitrogen supplementation and addition of organic acids. Although the addition of diammonium phosphate (DAP) reduced fermentation length, it did not guarantee the completeness of the fermentation process, suggesting that other factors could account for the reduced yeast activity in honey-must fermentations. Sixteen yeast-derived aroma compounds which contribute to the sensorial quality of mead were identified and quantified. Global analysis of aromatic profiles revealed that the total concentration of aroma compounds in meads was higher in those fermentations where DAP was added. A positive correlation between nitrogen availability and the levels of ethyl and acetate esters, associated to the fruity character of fermented beverages, was observed whereas the presence of potassium tartrate and malic acid decreased, in general, their concentration. This study provides very useful information that can be used for improving mead quality.

This work was carried out in the framework of project the PTDC/AGR-ALI/68284/2006

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Propagation of *Catharanthus roseus* (L.) G. Don by shoot proliferation

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Catharanthus roseus (L.) G. Don is one of the most important medicinal plants due the accumulation in the leaves of vinblastine and vincristine, which were the first natural anticancer agents to be clinically used. The high pharmaceutical value of these secondary metabolites allied to their low levels in the plant made of *C. roseus* one of the most studied medicinal species. However, no efficient protocol for transformation and regeneration is available, a fundamental tool that would potentiate basic research on *C. roseus*, and enable the development of strategies for genetic improvement.

Thus, the objective of this study was to optimize an efficient protocol for regeneration of *C. roseus*, to be utilized for genetic transformation. An efficient regeneration system of *C. roseus* was established, using mature embryos cultured in Murashige and Skoog (MS) medium supplemented with 5 µM TDZ. An intensive proliferation of shoots was obtained using MS medium supplemented with 4.44 mM BAP, and maximum rooting efficiency was observed in MS medium supplemented with 1.2 µM IBA. A high survival rate was attained during acclimatization of regenerated plants.

For the production of transgenic plants, optimization of a transformation protocol mediated by *Agrobacterium tumefaciens*, using fusions of a *C. roseus* class III peroxidase gene with the red fluorescent protein (RFP), under the control of the 35S constitutive promoter, was started. This work showed that mature embryos of *C. roseus* are an excellent explant for the regeneration of whole plants. This will contribute for the genetic improvement of a highly important medicinal species, with potential impact in the pharmaceutical industry.

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Differential expression of Ag-NORs and their epigenetic inheritance in Old Portuguese bread wheat cultivars

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Ribosomal RNA (rRNA) genes expression was analysed in 48 Old Portuguese bread wheat cultivars, using the sequential technique of silver nitrate staining and Fluorescent *in situ* Hybridisation (FISH) with the rDNA sequence pTa71 and genomic DNA from *Aegilops tauschii* Coss., as probes. Most of the cultivars presented six Ag-NORs per metaphase and the remaining, only four. After germination of the cultivars with four Ag-NORs in the presence of 5-azacytidine, the *minor locus Nor*-D3 was reactivated, suggesting the involvement of cytosine methylation in rRNA genes expression. Direct and reciprocal crosses between cultivars with four and six Ag-NORs were performed to evaluate the rRNA genes expression in the F_1 and F_2. At the F_1 we noticed three types of non-mendelian inheritance of NORs, including a female parent effect. The F_2 showed a pseudo-mendelian ratio of 3:1 which highlighted the sub-dominance of six Ag-NORs. NOR methylation patterns and indexes were evaluated by genomic Southern-blot with *Msp*I and the pTa71 probe. Parent cultivars with four Ag-NORs showed higher NOR methylation than those with six Ag-NORs [1]. The F_1 NOR methylation patterns were similar among them and were intermediate relatively to the parental values, suggesting epigenetic inheritance. NOR methylation patterns and indexes were transmitted to F_2, revealing an attempt to stabilize the number and methylation index of NORs. Globally, the differential expression of NORs among the parent cultivars was due to natural variation in the degree of cytosine methylation being epigenetically transmitted to the following generations.

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Acknowledgments
Work supported by the PTDC/AGR-GPL/65876/2006 project and the Ph.D. grant SFRH/BD/17348/2004, both financed by the Portuguese Foundation for Science and the Technology (FCT).

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Cat proto-oncogene *ERBB2* in spontaneous cat mammary tumours: sequence variants and Haplotype analysis

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Naturally occurring tumours in domestic animals, that share a similar environment with humans and therefore might be exposed to similar risk factors, have been recognized as an interesting opportunity for comparative oncology. Although spontaneous tumours in companion animals have been repeatedly proposed as appropriate and valid model for tumour systems, critical genetic and molecular information is still lack [1]. *ERBB2* is widely considered a key oncogene involved in HBC onset and progression. In humans and animals mammary tumours, tyrosine kinase oncogenes, as *ERBB2* gene, are activated and its amplification/overexpression is known to confer poor prognosis in HBC [1]. *ERBB2* gene overexpressed was detected in 15-25% of HBC cases and 55% of CMT samples [1]. Somatic alteration of *ERBB2* expression is well established in human breast cancer. In the present study, we detect and characterize cat *ERBB2* gene genetic variations in normal samples and in a heterogeneous CMT population. We used thirty six cat mammary tumours Formalin-Fixed Paraffin-Embedded Tissue (FFPET) samples (n=36) and thirty four frozen samples (n=34).

The genomic DNA extraction from the FFPET were performed according to the protocol previous described by Santos and collaborators [2]. We examined ERBB2 sequence variants at exons 17, 18, 19 and 20, encoding receptor transmenbrane domain (exon 17) and part of tyrosine kinase domain (exon 18 - 20). Some genetic variations were only detected in the tumour population. As far as we know, this is the first attempt to examine ERBB2 genetic variations in cat and, more specifically, in cat mammary tumours genome.

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Anethum graveolens hairy roots biotransformation and glycosylation capacity: the β-myrcene, (+)-camphene, R-(-)-carvone and S-(+)-carvone cases

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The biotransformation capacity of *Anethum graveolens* hairy roots cultures was studied by evaluating the influence of the addition of β-myrcene, (+)-camphene, S-(+)-carvone or R-(-)-carvone (25mg/l) in the morphology, growth and volatiles production. Hairy root cultures were maintained for 7 weeks in SH medium, at 24ºC and 80r.p.m. in darkness, as in [1]. Substrates were added 15 days after root inoculation. Root morphology was studied by scanning electron microscopy (SEM) [2]. Growth measurement, volatiles isolation and analysis, and glycosidic bound volatiles assessment was as in [1].

SEM observations showed that the root morphology was not influenced by the substrate addition. In some cases, the hairy roots growth was higher in substrate added cultures than in corresponding control cultures.

Falcarinol (1-59%), apiole (2-37%), palmitic acid (1-8%), linoleic acid (1-2%), myristicin (1-8%) and *n*-octanal (2-20%) were the main constitutive volatile components.

No new volatiles were detected after addition of β-myrcene and (+)-camphene, despite the decrease in the substrates amount over time.

Nine biotransformation products were detected after S-(+)-carvone or R-(-)-carvone addition. However, the relative amount of the new volatiles differed with the monoterpene enantioisomer added. Dihydrocarveol acetate was the main biotransformation product (21%) in the R-(-)-carvone added cultures, whereas *cis*-carvyl acetate was dominant (11%) in S-(+)-carvone added cultures. The relative amounts of the substrates and some of their biotransformation products showed a time-course decrease.

After the addition of β-glycosidase to the decoction waters, the analysis of the extracted volatiles showed that part of the substrates and their biotransformation products were glycosylated.

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Eucalyptus species essential oils composition from field grown plants and *in vitro* culture

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Eucalyptus is the third most representative forest species in Portugal, following cork oak and maritime pine. In addition to its use in pulp and paper industry, the fresh or dried leaves are traditionally used in Portugal, in the treatment of several ailments.

Sixteen *Eucalyptus* species grown at Mata Experimental do Escaroupim were assessed for essential oil composition, which were analyzed by GC and GC-MS, and evaluated by cluster analysis [2]. Furthermore, micropropagated *in vitro* cultures were established from selected *E. globulus* clones according to [1], and their essential oils isolated after one year in culture.

Essential oil cluster analysis showed a high correlation ($S_{corr} \geq 0.80$) among eleven species (*E. bosistoana, E. botryoides, E. camaldulensis, E. cinerea, E. cordieri, E. globulus, E. polyanthemus, E. radiata, E. saligna, E. smithii and E. viminalis*), mainly due to their high 1,8-cineole content (27-83%). *E. pauciflora* and *E. ficifolia* formed another highly correlated cluster ($S_{corr} \geq 0.74$), given the high relative amount of α-pinene in both species (82% and 44%, respectively). The essential oils from the remaining three species were dominated by citronellal (36%, *E. citriodora*), piperitone (40%, *E. dives*), and α-phellandrene (45%, *E. urophylla*). *In vitro* micropropagated *E. globulus* essential oils showed marked differences when compared to those isolated from field grown plants.

Essential oil yield and composition screening of other *Eucalyptus* species, as well as the establishment of *in vitro* cultures, may support models of development of high-yield producing species that are economically profitable, simultaneously promoting an ecologically responsible and sustainable management of Portuguese forest.

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Histopathological features of pine wilt disease on the stem of P*inus pinaster* seedlings

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Pine wilt disease caused by *Bursaphelenchus xylophilus,* the pinewood nematode (PWN), inflicts significant damage to coniferous forests. It was detected for the first time in Europe in 1999, on *Pinus pinaster* growing on the Setúbal Peninsula (30 km Lisbon, Portugal) [1]. Since 2000, Portuguese authorities have implemented several control measures, but in spite of all these efforts the disease rapidly spread throughout the country, and more recently to Spain, becoming a serious threat to EU pine forests. Despite considerable research on this disease [2], a detailed mechanism of PWN invasion and migration through the trees has not been described. To understand the host reaction during disease development, two-year old *P. pinaster* seedlings were inoculated with PWN and samples, harvested after three, five and seven weeks, were prepared, following standard methods, for SEM and histopathological studies.

The initial symptoms of the disease began 1-3 weeks after inoculation, but symptom development varied among specimens. At “the early stage” of infection, when no external symptoms were yet visible, PWNs were observed within the lumen of cortical resin ducts. During the “advanced stage” of infection, when external symptoms were plainly visible, severe tissue degradation occurred. Cavities with irregular boundaries developed from degraded ducts and surrounding parenchyma cells. PWN were present in the xylem resin ducts and their number increased markedly. As the disease progressed the cambial zone degraded and the cavities expanded and fused. At the seventh week after inoculation the number of nematodes has increased dramatically and all pine tissues are severely damaged.

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This research is partially founded by FCT under contract PTDC/AGR_AAM/74579/2006

ISSR molecular characterization and leaf volatiles analysis of *Pittosporum undulatum* Vent. grown in the Azores archipelago (Portugal)

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Pittosporum undulatum Vent. (Pittosporaceae) has been introduced in several countries as an ornamental or for protection against wind [1]. The presence of this species in the Azores archipelago has created a large problem of biodiversity erosion and in addition to future control measures, finding economic use for this species would be also desirable. As no systematic evaluation on the volatiles composition or molecular studies from individuals of *P. undulatum* grown on the Azores archipelago has been performed, this study (i) assessed the genetic variability of *P. undulatum* Azorean accessions by ISSR analysis, (ii) determined the volatiles chemical diversity, and (iii) evaluated the relationships between genetic and chemical polymorphism.

ISSR evaluation was performed based on a random sampling, comprising 77 accessions which included plants from all the Azorean islands. Leaf volatiles were isolated, from a total of 123 accessions, by distillation-extraction and analyzed by GC and GC-MS [2].

Molecular studies grouped the accessions mainly according to the geographical collection site, with some exceptions. Cluster analysis based on the leaf volatiles chemical composition defined three main clusters, not related to samples site collection; they were based mainly on the relative amounts of limonene (3-89%), sabinene (0.1-64%) and terpinen-4-ol (traces-43%). Clusters obtained from both molecular studies and volatiles composition were not superimposable and no correlation between ISSR markers and volatile oils could be drawn.

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Glandular trichomes of *Chamaecrista dentata* (Leguminosae, Cesalpinioideae): development, structure and histochemistry

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Chamaecrista dentata is an endemic small tree from the quartzite rock fields of Minas Gerais, Brazil. All plant body of this species is covered with a fragrant and viscid secretion, similar to a varnish. In this communication the structure, development and histochemistry of *C. dentata* glands were reported. Leaves and flowers were fixed in glutaraldehyde and prepared for scanning electron microscopy or embedding in resins for anatomy. The histochemical characterization of the secretion was carried out in fresh sections. At the tip of the glands, glistening and viscid globules are observed. As the glands originate from a single protodermal cell, they must be classified as trichomes. The trichome initial cell undergoes two successive anticlinal divisions forming four elongated cells in a row, which divide periclinally. The upper daughter cells, through a series of anticlinal divisions, give rise to an outer secretory cell layer from which a rostrum-like projection develops. The lower daughter cells divide irregularly and form a short stalk and a foot. Mature trichomes, described now for the first time for Leguminosae, resemble peculiar peltate trichomes with a chimney. They consist of a short stalk and a globular head which bears a filiform apical projection with four cells of diameter and several cells length. At the secretory stage a narrow lumen develops within the apical projection and the secretion, accumulated in this newly formed intercellular space, is suddenly released when the trichome projection tip breaks off. The histochemical characterization of the secretion revealed an oleoresin rich in flavonoid aglycones.

Acknowledgements:

Renata Meira is grateful to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasil) for a post-doctoral grant abroad (CNPq PDE/202478/2006/08) and acknowledges Centro de Biotecnologia Vegetal for the research facilities.

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