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Novel natural and synthetic compounds for treating hormone resistant tumors

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Abstract

Cancer is a leading cause of mortality worldwide. Despite significant advances in medical research, deaths due to cancer are continuously increase. Data from 2010 has shown the colorectal, breast, prostate and lung cancer as the most common in Portugal. Considering the North Region, prostate cancer and breast cancer are the most incident in men and women, respectively. Despite all the improvement in cancer early detection and treatment, the high mortality rates challenges the discovery of new anticancer agents. In the last years, several team members were involved in the study of the bioactive potential of natural and synthetic compounds at the Health and Environment Research Centre at School of Health of the Polytechnic Institute of Porto (CISA-ESS—P.Porto). From previous works, extracts isolated from plants and synthetic compounds, such as ionic liquids and quinoxalines, revealed an interesting bioactive potential as cancer drug. Following these previous approaches and considering the background of the team members in cancer research, the main goal of this project is to infer about the potential of these natural and synthetic compounds as anti-cancer drugs to common North Portugal cancer hormone-resistant tumors, namely castration-resistant prostate tumors and estrogen-independent breast tumors. To achieve such goal, the project has a multidisciplinary team with knowhow in natural compounds extraction, synthetic chemistry, cell culture, molecular biology, and oncobiology, that will carry out the following strategies: a) production and characterization of natural and synthetic compounds to be tested for anticancer potential by CISA members with experience in natural compounds extraction and compounds synthesis; b) *in vitro* assessment of the anticancer potential in prostate and breast cell lines in order to select the most promising compounds by CISA members with an extensive experience in cell cultures and cytotoxicity screening; c) *in vivo* assessment of the

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anticancer potential of the selected compounds using zebrafish as model. This activity includes a collaboration with consultants with a strong expertise in zebrafish model; d) identification of altered gene expression upon treatment with the selected compounds in order to infer about the molecular mechanism of action underlying their cytotoxic effects on tumor cells; e) ascertain of clinical usefulness of altered gene expression on human which might be used to predict therapeutic response and thus serve as biomarkers for clinical management and therapeutical guidance. For these two last activities, the project has Portuguese Oncology Institute (IPO-Porto) as a partner, due to the strong expertise in cancer, namely in PCa and BCa. With this approach, we expect to identify new anti-tumor compounds targeting the most common and incident tumors in the North Region of Portugal.

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Copromoting Institution: Cancer Biology and Epigenetics Group – Research Center, Portuguese Oncology Institute of Porto (CI-IPOP), Portugal

Keywords

Breast cancer, Prostate cancer, Ionic liquids, Quinoxalines, *Taraxacum hispanicum*

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Evaluating the effect of the Ionic liquid [C16Pyr] [Amp] in hormone-resistant tumors using an *in vivo* zebrafish assay

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Abstract

The NASYTHOR project, aims to study the potential of novel natural and synthetic compounds as anti-cancer drugs on hormone-resistant tumors. One of the objectives is the evaluation of the *in vivo* efficacy of the anticancer activity of the compounds using *Danio rerio*, zebrafish embryo as a model. In this work we describe the general methodology, advantages and disadvantages. Among a large range of advantages in the use of zebrafish in cancer research, the high level of genetic and physiologic homology with humans, including brain, digestive tract, musculature and vasculature can be highlighted. Also, the immature immune system of the embryos favors xenotransplantation of human cancer cells, and makes these animals a promising experimental model to tumorigenesis, angiogenesis, invasion and metastasis. The ionic liquid cetylpyridinium ampicillin [C16Pyr] [Amp] induces cytotoxicity in hormone-resistant breast and prostate cancer cell lines. To study the possible use of [C16Pyr] [Amp] as an anticancer, the toxicity towards zebrafish embryos should be evaluated first by an acute toxic assay that should be carried out following the OECD 236 Guideline. The test should include increasing concentrations of [C16Pyr] [Amp], selected within those required for an antitumor effect, that should be determined previously. A control treatment and a treatment with K₂Cr₂O₇ as a positive control should also be used. Following the acute assay with the fish embryos, and considering the toxicity results obtained, studies should proceed to evaluate the potential inhibitory effect of the [C16Pyr] [Amp] on breast and prostate cells injected in the perivitellin space of zebrafish. Culture prostate and breast line cells, selected for their sensitivity to [C16Pyr] [Amp] should be harvested and prepared for microinjection. Zebrafish fertilized eggs should be incubated for 48-72h in fish water, collected and dechorionated.

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Anesthetized embryos should be injected with the same cell number. After confirmation of the microinjection success, embryos will be transferred and incubated in [C16Pyr] [Amp] treatments prepared with fish water and previous selected [C16Pyr] [Amp] anti-tumor effective concentrations. With this work we intend to contribute to the development of cancer research, increasing the knowledge of the process of prostate and breast hormone-resistant tumor cells development in the living zebrafish embryo model.

Keywords

Ionic liquids, hormone resistant tumors, zebrafish model

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Natural and synthetic compounds as anti-cancer therapies for breast and prostate cancer

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Abstract

Breast Cancer (BCa) and Prostate Cancer (PCa) are the most prevalent tumors in females and males, respectively, being the main causes of cancer morbidity and mortality, worldwide. Currently, there is no available curative treatment for the hormone-resistant tumors, being the development of new therapeutic strategies using innovative anticancer agents imperative. Thus, the main goal of this work was to evaluate the anticancer properties of natural and synthetic compounds through *in vitro* assays.

BCa, PCa and non-tumor cell lines were treated with natural compounds, extracts of *Taraxacum hispanicum*, and synthetic compounds, ionic liquids and quinoxalines. Phenotypic assays were performed for evaluation of cell viability, cytotoxicity, apoptosis and colony formation. Only the ionic liquid ([C16Pyr][Amp]) and the alcoholic extract of *Taraxacum hispanicum* (EAL) are associated with significant decreased cell viability, with a high index of selectivity. Hence, the additional functional assays were performed only for these two compounds. When characterizing the mechanism of cell death, treatments with both compounds proved to sensitize cancer cells to apoptosis, while rarely induced cell death by necrosis. Moreover, the ability of colony formation was reduced in cell treated with both compounds, although a more pronounced effect has been observed in PCa cell lines.

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Overall, functional assays revealed that both [C16Pyr][Amp] and EAL have a significant antitumor activity. Nevertheless, these observations must be molecularly analyzed to allow the identification of the major cellular pathways affected by these compounds.

Keywords

Breast cancer, Prostate cancer, Natural compounds, Synthetic compounds

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Molecular impact of [C16Pyr][Amp] treatment on breast and prostate cancer cell lines

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Abstract

Prostate Cancer (PCa) and Breast Cancer (BCa) are the leading causes of cancer morbidity and mortality, worldwide, when diagnosed in advanced stages of the disease. Currently available therapies have limited curative effect, leading to the progression to highly aggressive hormone-resistant phenotypes. Thus, the development of new anti-tumor agents becomes imperative. Ionic liquids are organic salts with anti-neoplastic activity and have been studied in the pharmaceutical industry. Previous work of our team demonstrated that the ionic liquid [C16Pyr][Amp] has significant anti-tumor properties in PCa and BCa cell lines. However, the main cellular pathways affected were not characterized. Therefore, the aim of this work was to explore the molecular impact of [C16Pyr][Amp] treatment in order to identify relevant genes that have altered expression upon treatment and that can justify the anti-cancer effect observed in the *in vitro* assays.

The treatment effect was evaluated by using a custom expression array panel including several genes involved in cell cycle, apoptosis, DNA repair and mTOR or MAPK/ERK pathways. The comparative Ct method was used to calculate fold-difference in gene expression between cell line with and without treatment and genes with a fold change above 1 or below -1 were considered.

We were able to identify a panel of differentially expressed genes upon treatment with [C16Pyr][Amp] that will be subsequently explored by proteomic studies in human PCa and BCa tissues.

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Keywords

Breast cancer, Prostate cancer, Synthetic compounds, Molecular analysis

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Production and characterization of natural and synthetic compounds for treating hormone resistant tumors

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Abstract

From previous and on-going studies, natural compounds/extracts isolated from *Taraxacum hispanicum* (1), Ionic Liquids (ILs) based on ampicillin and quinoxalines emerged as potential anticancer. Although described as potentially antitumor compounds, the mechanisms undergoing cytotoxicity remain unknown. Therefore, production of these kind of compounds were carry out in order to have a better understanding of their bioactivity.

Several approaches were made according to the type of extracts/compounds that were studied. In the case of the Ionic Liquids Based on ampicillin (2), the buffer neutralization method was the methodology that we used to synthesize and that was developed and already described by us (2). The purity of the compounds was determined by ¹H and ¹³C Nuclear Magnetic Resonance and mass spectrometry. Regarding quinoxalines, the compounds were purified by reduced pressure sublimation, and thermal stability was verified by DSC (3). On the topic of the natural compounds, the leaves of *Taraxacum hispanicum* were collected from a producer in Vila Nova de Gaia (Portugal), and authenticated by a specialist. A characterization of the extracts was performed by High Pressure Liquid Chromatography (HPLC) and Diode Array (DAD), according to Schütz et al (4).

As expected the products were obtained pure, with good yields and in enough quantity to carry out the functional studies with cancer cell lines.

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Keywords

Ionic liquids, natural products, quinoxaline, hormone resistant tumors

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Novel biosensors for venous thromboembolism in oncology: the STRIP2SENSE project

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Abstract

Cancer-related venous thromboembolism (VTE) worsens disease prognosis, leading to death in a vast majority of cases. A point-of-care identification of VTE for risk-assessment and treatment prescription could enhance the quality of life and survival of cancer patients. Thus, the development of biomedical devices for low-cost and rapid monitoring of VTE biomarkers is imperative.

The versatility of biosensor approaches can answer the demand for more accurate and personalized portable devices. New nanotechnological-based strategies are particularly valuable concerning the detection of disease biomarkers in complex biological samples, like body fluids containing interfering molecules. Molecular imprinting is a cost-effective technique to obtain selective recognition of target biomarkers. The synthetic complementary recognition nanocavities in polymeric matrices show advantages over their biological counterparts, like improved stability and reusability.

The new technology proposed in the STRIP2SENSE project is based on non-invasive test strips tailored with biomimetic plastic antibodies that assure the required specificity, sensitivity and accuracy in real-time analyses. It is expectable that the novel sensing strips compete with the standard methods and can have future translation into clinical practice.

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Artificial antibodies based potentiometric sensors for monitoring diabetic ketoacidosis

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Abstract

Diabetic ketoacidosis is a pathological condition characterized for the complex disordered metabolic state (as hyperglycemia, metabolic acidosis, dehydration or ketosis), caused by the total failing of insulin production for beta cells in the islets of Langerhans (type 1 diabetes) or abnormalities in the peripheral insulin action and insulin secretion (type 2 diabetes). This pathogenesis can be also associated with the increase of counter-regulation hormones, leading to an increase in the hepatic glucose synthesis and a decrease in the peripheral tissues, resulting in hyperglycemia and hyperosmolarity. The effect of lipolysis increase leads to an increase in the production of free fatty acids, which are oxidized in the hepatic microsomal system and converted to acetyl-CoA. When acetyl-CoA production exceeds hepatic utilization capacity, this substance acts as a substrate for the production of ketone bodies (β -hydroxybutyrate (BHB), acetoacetate and acetone), causing ketonemia and metabolic acidosis.

The present work describes an original approach to create a potentiometric biomimetic sensor for BHB detection. For this purpose, a molecularly imprinted material, acting as an antibody, was obtained by bulk polymerization of acrylic acid, trimethylolpropane trimethacrylate and BHB. In parallel, a non-imprinted polymer material (NIP) was produced, following an equivalent procedure, without the target template. The chemical features of the obtained MIP materials were followed by Fourier Transform Infrared (FTIR) spectroscopy. The selective membranes were prepared by dispersing the sensing material in a plasticized PVC membrane, including or not a lipophilic ionic additive. These membranes were then casted on a solid conductive support made of graphite, placed at the smaller end of the plastic body of an insulin syringe. The analytical

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performance of the devices showed sensitive readings of BHB in wide concentration range, up to 0.01 mg/mL.

Overall, the proposed biomimetic sensor offers a simple and low-cost approach for monitoring BHB in the diabetic ketoacidosis disease, being the application to real serum samples successful.

Keywords

Diabetic ketoacidosis, β -hydroxybutyrate, Molecularly imprinted polymers, Potentiometry.

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Molecular imprinting technology for detecting biomarkers of venous thromboembolism

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Abstract

Innovative non-invasive sensing devices to be used in point-of-care are foreseen to benefit patients and simultaneously improve the management of healthcare resources. Coagulation processes are highly linked to cancer, and the increased risk of developing venous thromboembolism (VTE) is a clinical concern. Cancer patients with VTE have worse survival outcomes. Thus, it is crucial to have an early and accurate VTE diagnosis.

Herein, a new technology is proposed to detect biomarkers of VTE, helping to solve the limitations of the current diagnostic techniques. The biosensor relies on high-affinity molecularly imprinted polymers (MIP), which guarantee selective and sensitive biomarker recognition. The sensing layers shall be assembled on test strips that hold the obvious advantage of providing a rapid and non-invasive access to urine biomarkers in single-use point-of-care.

The MIP-based sensors were designed and synthesized as selective recognition elements of peptides of the coagulation cascade. The analytical performances enabled to obtain sensitive responses in the range of physiological relevant levels. Thus, the proposed straightforward approach envisions a rapid development of the aimed test strips.

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BIO4DIA: Early detection and monitoring of metabolic progression of type 2 diabetes

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Abstract

According to the latest International Diabetes Federation 2015 report, the total health expenditure with type 2 diabetes (T2D) spent globally was of 673 billion USD in 2015 and it expected that this effort increases up to 802 billion in 2040. Additionally, patients experienced a series of complications, ranging from eye, cardiovascular, and kidney disease as well as pregnancy complications, nerve damage, foot infections and impaired regeneration [1-4]. Based on the most recent research in this area the cornerstone of treatment of T2D is the adoption of a healthy diet, increased physical activity and maintenance of a normal body weight and those are modifiable risk factors [5-9].

Actually, the large investment in T2D research allowed the identification of biomarkers that can be used to describe the progression of a sub-clinical stage to a clinical stage of diabetes and some biomarkers have been described with predictive potential value to differentiate between progressors/non-progressors [10-12].

Nevertheless, there is no technology at the present moment, that could be used at bed side and that could be rapidly and easily used by clinicians to monitoring their high-risk patients and also that could be sensitive, precise and cost effective. So, smaller, faster (one-step), and cheaper devices are highly desired for replacing the time-consuming and expensive laboratory-analytical methods. Biosensors meet these criteria. The idea behind the present proposal is to develop a point-of-care (POC) device for the measurement of biomarkers with predictive potential value to differentiate high risk individuals between progressors/non-progressors [13-17].

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This new perspective would benefit all the interested parties: Patients would benefit from better medical care in a personalized medicine perspective; Clinicians would benefit from a rapid, accurate, sensitive, low cost and easy-to-use tool for diagnosis and monitoring; Global health would benefit specially in those cases where early detection could reverse the course of the disease, decreasing the direct and indirect costs associated to that.

The BIO4DIA research team has know-how in the field (ADPD the reference institution for Diabetes health care in Portugal, Biomark/P.PORTO a leading institution for biomarkers research and biosensor development) and the working conditions to develop a prototype of POC device to measure T2D biomarkers (Technology readiness levels at stage 3/4), with future Patent submission.

To achieve these outcomes the project will take into account the following stages: 1) the reference values adjusted for the Portuguese population of the selected biomarkers must be clearly identified; 2) The biorecognition element of the biosensor must be selected in a way that allows the high-selective and accurate for the proper measurement of the selected biomarker; 3) The prototype should be submitted to a pre-test in a clinical environment.

This project is being developed with the collaboration of academic institutions that have the opportunity to involve both researchers and students from different BSc, MSc and PhD backgrounds that are taught in house: BSc in Medical Biotechnology; Biomedical Engineering, Chemical Engineering, MSc in Computation and Medical Instrumentation, Chemical Engineering and Health Biochemistry and from the PhD in Biotechnology (col. with Vigo University/Spain). The clinical community of APDP have the opportunity to involve several health professionals such as doctors from several specialities regarding Diabetes and their complications, nurses and health technicians.

Finally, the BIO4DIA team is committed in prevention of T2D. Regarding this topic, it is intended to develop several workshops and health education programs. APDP has a School of Diabetes with programmes for both professional and general publics for diabetes education in Lisbon and Oporto.

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Keywords

Type 2 Diabetes; Early Diagnosis Biomarkers; Point of Care.

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Glycated Albumin: A new biomarker for monitoring metabolic progression of diabetes

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Abstract

One of the consequences of the metabolic progression of diabetes is kidney disease, being also a main cause of diabetes related morbidity and mortality. Therefore, earlier adoption of methods to estimate chronic kidney disease risks along with a more accurate glycemic index for patients with type 2 diabetes mellitus is highly desirable.

One of the widely used tubular injury markers is N-acetyl- β -D-glucosaminidase (NAG), a lysosomal enzyme of renal proximal tubular epithelial cells. One study demonstrated that the association between glycated albumin (GA) and uNAG excretion and observed that GA was significantly associated with uNAG excretion independent of other confounding factors. Consequently, this study suggest that GA can be a strong independent predictor of early renal tubular damage, beyond its role as a surrogate marker of glucose control (Huh et al., 2018).

The glucose and other sugars react spontaneously with free amino terminal residues of serum proteins, like albumin (the most abundant of serum protein). Glycated albumin (GA) levels increase in states of abnormally high glucose concentrations such as diabetes and can hence be used for assessing glucose control over a short to intermediate time frame (approximately 14-21 days, half-life of serum proteins (Danese et al., 2015).

Glycated albumin (GA) has been identified as a good biomarker because are not affected by changes in erythrocyte lifespan, measurement is not influenced by anemia or other conditions which invalidate HbA1c measurements (Wu et al., 2016). Several studies revealed the association of increased GA values with the presence of diabetic retinopathy, nephropathy, and cardiovascular

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complications; these findings also support the use of GA measurements in the diagnosis of diabetes (Danese et al., 2015; Wu et al., 2016).

Although further studies are needed to establish whether GA may complement (or even replace) conventional measures of glycemic status such as HbA1c, it is undeniable that GA is already helping the clinical management of patients with diabetes in whom HbA1C values are unreliable (Danese et al., 2015).

Keywords

Type 2 Diabetes mellitus; Early Diagnosis; Glycated Albumin

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Procalcitonin: a marker for predicting the risk lower extremity amputation in infected wounds

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Abstract

Diabetes Mellitus is a metabolic disorder that is growing at epidemic proportions worldwide and is consensually classified in type 1 Diabetes, type 2 Diabetes and Gestational Diabetes (1). One of the most common complication of diabetes is the lower extremity is the diabetic foot ulcer (DFU). Diabetes is also the most common cause of lower extremity amputation (LEA). Several foot disorders, such as foot ulcerations and infections are a major source of morbidity and a leading cause of hospitalization for persons with diabetes. Thus, ulceration, infection, gangrene, and limb amputation are major complications of the disease, estimated to cost billions of dollars each year and have attracted the attention of health policy providers (2, 3).

The most common single precursor to lower extremity amputations among person with diabetes is the foot ulceration and the treatment of the infected foot wound represents one quarter of diabetic hospital admissions. The risk calculation and prediction LEA rely on stratified systems to be applied in the clinical practice. The several clinical valuable systems used to stratify subjects with DFU by risk of consequent LEA present overall substantial accuracy values. However, authors consider better for implementation in daily clinical care those that use fewer easy to use dichotomic variables (4,5).

Decision-making tools for the management of lower extremity infections are typically depend on inflammatory markers. Such markers rely basically on the white blood cell count, erythrocyte sedimentation rate, and C-reactive protein which are used to earlier assess DFU patients and/or to monitor the progression of medical or surgical therapy. Initially, procalcitonin was used to monitor

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antibiotic therapy patients in the intensive care setting but now procalcitonin as being revealed useful as a new inflammatory marker that is specific for an infectious course (5,6).

In the present project we pretend to evaluate the predicting value of procalcitonin as biochemical marker for the prediction of risk of amputation and study its significance as an additional parameter for a clinical estimation tool regarding risk assessment.

Keywords

Procalcitonin; Type 2 Diabetes; Lower Extremity Amputation (LEA); LEA Risk Assessment; Diabetic foot infection;

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Galectin-3: a biomarker of metabolic progression of Type 2 Diabetes?

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Abstract

According to the International Diabetes Federation, Diabetes mellitus type 2 (DM2) has affected more than 425 million people in 2017 worldwide [1]. Not only is it a disease responsible for more than 4 million deaths, but also a trigger for other non communicable diseases and a huge burden on national health systems.

The most important clinical manifestation of T2DM is hyperglycemia and monitoring of blood glucose levels remains the only method of screening. However, when glucose levels are high, the disease is already in place. The large investment in DM2 research allowed the identification of biomarkers that could be used to describe the progression of diabetes and some were described as having a predictive potential value to differentiate between progressors / non-progressors. One of the biomarkers described is Galectin-3 (Gal3). Previous studies of our group have already demonstrated a correlation of Gal3 between diabetic and non-diabetic animals [2].

Gal3 has multiple functions depending, among other factors, on its location and target tissue. Intracellular Gal3 acts as a pre-mRNA splicing factor and regulates the cell cycle through the modulation of cell proliferation, death and differentiation. In addition, it promotes cell proliferation and cell survival, protecting against induced apoptosis. Extracellular gal3 further regulates cell adhesion. Gal3 is capable of binding high affinity, internalization and degradation of advanced glycation products, playing an important role in diabetes and aging [3].

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Pugliesi et al. demonstrated that in diabetic cohorts, plasma levels of Gal3 were correlated with the prevalence of diabetes and related metabolic conditions. Galectin-3 is considered not only as a marker of heart failure but also as a mediator of the disease due to its pro-fibrotic action [4].

The aim of the present study is to evaluate the reference values for non-diabetic, pre-diabetic and human diabetic individuals of the Portuguese population.

Keywords

Type 2 Diabetes; Early Diagnosis; Galectin-3.

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Diabetes Risk Assessment: Clinical Screening Tools

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Abstract

The epidemic of type 2 diabetes (T2D) continues to soar worldwide. Driven by changes in human habits, from availability of energy dense processed foods to the decrease of physical activity, T2D constitutes already a heavy burden of disease and a worrisome barrier for the sustainability of National Health Systems. Indeed, it affects 415 million people (8.8% prevalence), was responsible for 5.0 million deaths in 2015, and is estimated to cost 1/9 of all Health expenses (1).

Within Europe, Portugal is reported as having the highest prevalence of diabetes: an estimated 9.6% by World Standard Population (2). But if we take into consideration the age distribution of the Portuguese population, then estimates increase to 12.9%. This means that 1 million Portuguese have diabetes, with almost half of these still undiagnosed. Additionally, 2 million (a third of the adult population) are estimated to have intermediate hyperglycemia (or prediabetes), being largely unaware of this condition (3).

Since it was clearly shown that a life style modification program was able to prevent, over 3 years, more than half the expected new cases of diabetes (4), many clinical trials have provided evidence on the beneficial effect of interventions targeting individuals at high-risk and focused on nutrition and exercise (5). These in turn have supported the proposal of guidelines for diabetes prevention (6,7). Nonetheless, the main challenge remains to effectively translate these clinical trials and guidelines to widespread lifestyle interventions (7).

Portugal is in dear need of a strategy based on prevention for managing the diabetes crisis. That was recently highlighted in ‘A Future for Health’, a report commissioned by the Calouste

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Gulbenkian Foundation, where Diabetes Prevention was elected one of three main challenges to preserve SNS sustainability (8).

Regarding diabetes risk assessment, a large number of tools have been proposed to detect people at high- risk to develop diabetes and/or already within prediabetes (9-11). Several are based on questionnaires of parameters obtained noninvasively (12), and, among those, the Finnish T2D Risk Score (FINDRISC) has been validated and used as a screening tool for diabetes prevention programs (13-15). The FINDRISC 8 item questionnaire structure enables it to be applied in primary care, field screening initiatives, or even by individuals themselves. In Portugal, it has been used by us in the national diabetes prevalence study (PREVADIAB) (16). A supplemental strategy is to identify high-risk by screening existing Electronic Health Records (17).

In this work we propose to compare four risk assessment tools in their easiness of application and reliability, thus allowing stratification the risk of metabolic progression of the disease.

Keywords

Type 2 Diabetes mellitus; Prediabetes; Diabetes Risk Assessment; Diabetes risk score.

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Gradual Loss of the Ability of Glycated Haemoglobin to Assess Total Serum Protein Glycation in a Population Study

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Abstract

Objective: It has been described that fructosamine is directly proportional to glycated haemoglobin (HbA1c), and that their correlation can be expressed by the equation $\text{Fructosamine} = (\text{HbA1c} - 1,61) * 58,82$. Since it has been proposed that serum proteins, other than haemoglobin, when exposed to glycation may be directly related to a further decrease in insulin sensitivity, we investigated, in a general Portuguese population, if glycation levels as measured through HbA1c can be confidently extrapolated to express total protein glycation.

Results: HbA1c and Fructosamine measurements showed weak correlation ($r^2=0,2584$) in the studied population. Additionally, it was observed that this drift is independent of the classification of glucose metabolism provided both by fasting glycemia and 2h post-OGTT. Paradoxically, when the variation between measured and theoretical (estimated through HbA1c) Fructosamine was analysed by Fructosamine quartiles, it was observed that while for the first quartile there was a good correlation between HbA1c and total protein glycation, this is gradually lost until the last quartile, where extrapolation based on HbA1c underestimates the real measure of serum total protein glycation in about 30% ($p < 0,001$).

Conclusion: HbA1c constitutes an inadequate surrogate for assessing serum total protein glycation, at least in the general population studied. Thus, to study the glycation of other serum proteins other than haemoglobin should be use specific quantification tests to determine glycation levels in those proteins.

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Keywords

Total serum protein glycation; Glycated haemoglobin; Glycation gap; Glucose management; Biomarkers; Type 2 Diabetes

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FishBioSensing: an innovative electrochemical biosensor for quality control of fishery products

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Abstract

One of the objectives of the FishBioSensing project is the development of an electrochemical biosensor for the determination of the safety and quality of fishery products to address the current fish industry demands. Electrochemical biosensors are good alternatives to conventional methods since they provide fast and simple detection without the need of specialized workers, complicated sample treatment procedures and expensive equipment. The chosen analyte in this work was histamine, which is an important fish freshness indicator; inappropriate storage conditions after fishing increases its levels. Furthermore, the consumption of products with a high concentration of histamine can cause food poisoning, so its maximum concentration is legally regulated.

Histamine was analysed in fish samples by using a simple, miniaturized and low-cost enzymatic sensor based on a screen-printed electrode. The sensor was constructed by immobilizing an enzyme (diamine oxidase) on the surface of the electrode and histamine was quantified through the use of a redox mediator to record the analytical (amperometric) signal.

The sensor showed a selective response towards histamine and provided accurate and precise results and a limit of detection that meets the needs of the fishing industry. Moreover, its response maintained stable for over a month after construction. Therefore, the developed sensor could be useful in routine analysis.

Keywords

electrochemical biosensor; histamine; food safety and quality; fishery products.

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Steam gasification of glycerol and animal fat

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Abstract

Residues are an endogenous and renewable energy source, which valorisation is imperative. The national energy strategy in 2020 made a commitment to renewable energies, providing strategies to improve the use of the energetic potential of waste recovery. The growth of biodiesel industry in Portugal has generated large amounts of crude glycerol. The current solution is the sale of this contaminated by-product which is no longer an economically interesting option. On the other hand, the resulting animal fat from leather industry has special characteristics, which determine its application and management. So, there is a real concern related to the management of those by-products. This project aims to evaluate the technical viability of crude glycerol gasification and glycerol/fat mixtures co-gasification. Crude glycerol was pre-treated using an ion exchange process, in order to reduce its salt content. The gasification process was studied in a fixed bed reactor using steam as gasification agent. The effect of temperature, oxidant agent and glycerol/fat ratio on the producer gas composition were analysed. The results revealed that the gasification of treated glycerol and the co-gasification of glycerol/fat mixtures appear to be feasible options for the valorization of these by-products. Best result of gasification parameters were obtained at the highest tested temperature (950°C). The results showed that the increase of the water content in the feed mixture lead to higher values of H₂ in the producer gas. The parameters that assess the gasification performance revealed very good results, similar, in magnitude, to the ones obtained for technical glycerol gasification.

Keywords:

crude glycerol, co-gasification, steam reforming, fixed bed

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Oil Spill Mitigation with a Team of Heterogeneous Robots

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Abstract

Marine oil spills have a large economic and ecologic impact in the ecosystem of marine life. Incidents with oil spills occur with some regularity during the exploration, production, and transport of petroleum products[1]. In the last nine years, between 2010 and 2018, were registered 59 spills with 7 tonnes or more, resulting in a total of 163.000 tonnes of oil spills in the environment, with 92% of this amount was spilled in 10 incidents[2]. The occurrence of such incidents requires immediate, simple, effective and eco-friendly actions to minimize environmental damages. First-line responses typically include physical (e.g., controlled burning; absorbing) and chemical (e.g., dispersing) removal of oil, which is largely constrained by maritime conditions. Though these treatments are important to rapidly control the diffusion and drift of the oil, they are not suitable for ecological restoration. The use of microorganisms with a natural capacity to degrade petroleum is highly advantageous in an environmentally friendly process and allows complete decomposition of complex petroleum hydrocarbons[3]. The success of bioremediation, to improve petroleum removal and reduce clean-up time and cost, relies in two major approaches: (i) addition of nutrients to stimulate the growth of the microorganisms that break down oil (Biostimulation) and (ii) the addition of pre-grown microbial cultures/consortia to enhance microbial populations (Bioaugmentation). This mitigation strategy usually involves the introduction of non-native microorganisms in the affected environment. The ROSM (Robotic Oil Spill Mitigation) project proposes to address the development of autonomous and coordinated actions able to increase the efficiency of the bioremediation process, by deploying microorganisms and nutrients in oil spill detected areas with a team of heterogeneous robots. The proposed approach will be based on the production of native microbial consortia with bioremediation

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capacity, and the adaptation to autonomous vehicles for in-situ release of autochthonous microorganisms (bioaugmentation) and nutrients (biostimulation), and their use combined with heterogeneous robots, autonomous surface vehicle (ASV) ROAZ and unmanned aerial vehicle (UAV) STORK, with different levels of intervention[4], will result in a more cost-effective manner, without risking human lives. Preliminary field tests have been performed in Leixões Harbour in Porto, Portugal, with an oil spill created inside Gazebo simulator scenario, projected on a known position from the real world. Both vehicles were able to coordinate the oil spill mitigation task with the ASV ROAZ being able to contour the oil spill while is deploying microorganisms and nutrients (bioremediation) capable of mitigate and contain the oil spill spread, and the second one for an UAV responsible for performing the coverage of the entire spillage area with the same microorganisms and nutrients deployment capabilities.

Keywords

Oil Spill, Mitigation, Bioremediation, Heterogeneous Robots, UAV, ASV

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Development of a MultiRotor UAV for Oil Spill Mitigation

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Abstract

Over the last few years, oil spill incidents occur with some regularity during the exploration, production and transport of the petroleum products, causing a large economic and ecologic impact in the world community, with high losses in the marine ecosystems[1, 2]. In 2018, the total volume of oil lost to the environment recorded was approximately 116,000 tonnes[3], causing huge impacts into the ecosystems. Beside this, current oil spill cleaning technologies aren't suitable for ecological restoration neither fast enough to start the mitigation process, since this operation requires several surface and aerial vehicles. To minimize these impacts and reduce the time response of the initial mitigation process, autonomous vehicles, such as UAVs and ASVs, can be used to perform oil spill monitoring and mitigation tasks[4]. With this thought, ROSM and Spilless projects emerged, aiming the development of an autonomous and coordinated system between two vehicles, UAV and ASV, in order to increase the efficiency of the mitigation process using bioremediation techniques[5]. This paper presents the design and implementation of the multirotor UAV developed for both projects, capable of identifying, locating and mitigating the oil spill, by using a release system of consortia with bacteria and nutrients. In this way, all mechanical and electronic aspects were developed, resulting in a new hexacopter platform with a novel design. The vehicle is composed by a set of visible and infra-red cameras, a LiDAR sensor, an autopilot, a high precision IMU and GNSS, a high power on-board computer, used for processing all the data and perform an autonomous control, and a bacterial spreading system, presenting an overall payload of 45 kg. Several flight tests were carried out, in indoor and outdoor environments, allowing to adjust the control parameters of the UAV and the release system behavior in the presence of different conditions of operation. The preliminary tests of the projects occurred in the Leixões Harbour in Porto, Portugal, where the oil spill was implemented in a simulated

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environment, resulting in a cooperative and simultaneous maneuver between the UAV and the ASV.

Keywords

Oil Spill Mitigation, Bioremediation, UAV

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Cooperative Maneuver for Oil Spill Mitigation with a Team of Robots

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Abstract

The paramount importance of obstacle avoidance in a path-planning function, for an autonomous mobile robot, is a well stated fact. In a two-dimensional scenario, that contains obstacles with an arbitrary shape/contour and well-defined and continuous boundaries, the vehicle should be capable of defining a trajectory from its initial position to the goal position, while maintaining a safety distance from the boundaries of the obstacles present in its path, to avoid a collision with said obstacles. This work presents a comparison study between Obstacle Avoidance algorithms using Potential Functions and using Local Path Planning, more specifically between two approaches presented in [1] and in [2]. This comparison and following presentation of comparative results are part of an INESC TEC study of algorithms to be applied, under the national research project ROSM (Robotic Oil Spill Mitigation). This project aims to develop new and viable solutions to tackle maritime pollution, in particular oil spills, through the production of native organisms, with bioremediation capacity, and the adaptation of unmanned and autonomous vehicles for the application of such bioremediation treatments. By doing so, these systems can be used as first line responders to pollution incidents in a fast, efficient and low cost way.

The specific scenario for the application of this obstacle avoidance algorithm is an ocean surface with an Autonomous Surface Vehicle (ASV), ROAZ [3], that has an aerial perspective of the scene. The oil spillage represents one or multiple obstacles in two dimensions while the vehicle knows its position provided through its sensors, GPS and IMU and knows the obstacles positions through the aerial perspective provided by an auxiliary Unmanned Aerial Vehicle (UAV). The vehicle should be able to contour the oil spill completely, while maintaining its orientation face to the closest obstacle contour point, to have its sprinkler facing the spillage and release the spillage

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containing chemicals. The results will detail the evaluation of several methods[2][1] able to ensure a robustness and efficient approach for oil spill mitigation with an ASV.

Keywords

Oil Spill Mitigation, Path Planning, Cooperative Perception, Bioremediation, UAV, ASV

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Tourism and Heritage Routes including Ambient Intelligence with Visitants' Profile Adaptation and Context Awareness

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Abstract

The aim of the project TheRoute is to generate automatic routes for tourists and visitors to points of interest related to Tourism, Heritage, Arts and Culture. The Platform developed in Porto creates customized tourist routes in the North region of Portugal. The suggested routes are appropriate to the profile of the tourist and groups of tourists. The project also considers aspects related to mobility (transportation), health, well-being and accessibility. The system is accessible by computer, tablet and smart phone environment, covering the life cycle of the tourist experience. TheRoute has been developed considering the notion of Ambient Intelligence (AmI) and Context Awareness. This paper focuses on the proposed Architecture, highlights the interface of the mobile platform, the Modelling process of Points of Interest and the Thematic Tourist Tours.

Results and conclusions

The development of TheRoute was driven by a multidisciplinary collaboration between three Higher Education Institutions (P.Porto, ISEP, through the GECAD R & D group and the Polytechnic Institute of Viana do Castelo) and one Enterprise (Douro Azul), based on an extensive literature review about mobile applications for tourism. This process was also a user (Tourist) participatory design approach which advocates a change of perspective: instead of design for users, it is projected to design with users.

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The mission of theRoute was to make an easy and automatic recommender system for tourist to visit the North region of Portugal. Also, the platform can promote the marketing of the Regional Tourism and Monitoring Information in an Intelligent way. TheRoute Systemic capable of understating the tourist information and convert it as a targeted suggestion for his/her experience.

We designed one multidisciplinary Ambient Intelligent system for getting appropriate information, based on tourist profile, psychology, available Point of Interests and also with related information. Additionally, with the use of the system and its statistics, it will be possible to analyse the profile of the tourist (or group of tourists) of an area, identifying touristic necessities in order to improve the design of touristic-related offers.

Also, the solution to presents several thematic tours route to the end user can, in the long run, allowing an approach to different Heritage typologies, by identifying potential objects/motifs/elements of interest (POIs) of the North region of Portugal.

Keywords

Tourist Route Generation, Touristic Profiling, Recommender System, Thematic Tourist Tours

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The influence of external factors on the energy efficiency of public lighting

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Abstract

LED-based technology is transforming public lighting networks, favoring smart city innovations. Beyond energy efficiency benefits, LED-based luminaries provide real time stateful data. While most of the municipalities manage all their luminaries equally, independently of its state or the environmental conditions, we aim to go beyond basic consumption monitoring and dimming control: we combine the individual luminaries' state data with data about the environment (e.g. temperature, humidity) to model, at an individual level, power consumption and working temperature. These two variables are of utmost importance for two major challenges addressed by this project: power consumption and preventive maintenance. We thus built two different models (distributed random forest) for predicting power consumption and working temperature. These models evidenced two relevant aspects: 1) the operation of the luminaries is significantly influenced by external factors (namely environmental ones); and 2) there are operational variations between luminaries, that is, each one operates at a different temperature and consumes power differently. The main contribution of this work is to model these aspects, that must be considered when developing more precise energy consumption predictions or defining preventive maintenance plans.

Keywords

Public Street Lighting; Random Decision Forests; Energy Efficiency

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A Decision-Support System for preventive maintenance in street lighting networks

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Abstract

Smart lighting networks are gaining relevance in modern cities as traditional systems are replaced by LED ones. In their management, maintenance is one of the central tasks. Maintenance plans can be implemented in two ways: preventively (by continuously monitoring and predicting failure) or reactively (by acting upon failure). We propose an approach for preventive maintenance that relies on the combination of two complementary models of the network, that classify the state of each luminary as normal or abnormal: one is based on rules defined by a Human expert, the other is based on a statistical analysis of the data. Both models have an accuracy of >0.99 , showing that it is possible to accurately distinguish between normal and abnormal states. Moreover, both models have a high degree of concordance in their predictions. Based on both models, a classification service was developed that can be used in real-time to detect luminaries that are working abnormally. This service is the basis of a Decision-Support System that helps human experts identify luminaries that operate abnormally and are thus more likely to fail, improving the efficiency and efficacy of preventive maintenance plans.

Keywords

Public Street Lighting; Preventive Maintenance; Decision Support System

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Innovative Consumer Aggregation to Improve Demand Response and Tariff Design for Energy and Services Transactions

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Abstract

In CONTEST project, business models and market structures for intensive and efficient use of distributed energy resources in local energy communities, considering the interaction with current and future wholesale markets have been explored. Aggregation of consumers and of energy resources have been addressed as enablers for the participation in local transactions, and in wholesale markets. Demand response and the active contribution of consumers are incentivized, namely through innovative adequate tariff and remuneration. A hybrid simulation platform combining multi-agent technology with physical emulation was improved, enabling the realistic test and validation of the results.

Keywords

Aggregation, demand response, remuneration, smart grids

Results

The results obtained cover the following aspects:

- Business models to support the implementation of a fully transactive energy system, through the integration of local electricity markets in the current market structure.
- Innovative aggregation methods using Artificial Intelligence and data mining techniques.
- Definition of advanced dynamic energy tariffs and remuneration schemes, which will transmit the local and wholesale market value to the small players.
- Validation of the developed business models and methods in a laboratorial prototype, through the integration in a simulation platform existing in ISEP.

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Some of the publications resulting from the project:*Published in journals:*

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SmartOrthosis - Automatic Clinic Measures and Comparison of Heads using Point Clouds

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Abstract

Positional plagiocephaly is a condition characterized by an asymmetrical distortion of the cranium. A baby's skull is not strong, so it can suffer deformations, which cause plagiocephaly. In severe deformations, to fix the problem, the baby may need to use an orthosis, that should be used when the baby has between 3 and 18 months of age [1].

The orthosis will allow the head to grow gently back into a normal shape. This treatment must be done early, before the fontanelles close and the cranium fuses together.

The SmartOrthosis project aimed to automatize the process of orthosis' creation for the treatment of positional plagiocephaly. The development of SmartOrthosis was driven by a collaboration between ISEP (from P. Porto), through the LAMU (Multimedia Lab) team and IPCA, through 2AI.

In order to reach the aforementioned objective, the development was divided into three phases:

- automatic anthropometric analysis,
- automatic cranial orthosis' design and
- cranial orthosis' prototyping.

In the phase of the automatic anthropometric analysis, it was developed methods to treat the point clouds in order to obtain the necessary measurements and points. The automatic cranial orthosis' design will develop processes to create the 3D model of the cranial orthosis, using the measurements obtained previously.

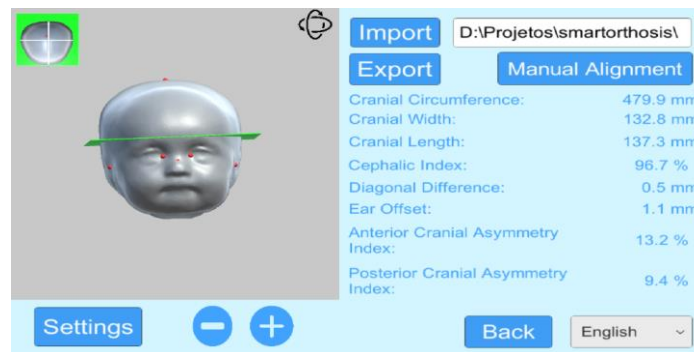
The prototype of the orthosis will be made using the previously obtained models and it will be studied materials and sensors necessary for a correct functioning of the orthosis. In order to make the results

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usable for doctors and other medical professionals, a solution with a graphical interface has been developed by ISEP team.

The solution was implemented using Unity, C# and R. The Unity was chosen by its capabilities in building interfaces that conjugate 2D and 3D elements and because Unity allows to see the results more easily and fast while the solution is developed. The R language was chosen because of the diversity of processing algorithms already developed in this language.

The graphical interface of the developed solution has two main areas. The first area is dedicated in the visualization and manipulation of point cloud. The second area is dedicated to view the results and make the necessary operations. In the Measures functionality, it's shown at left top corner the top view of the head. The top view of the head allows the user to see where the width and length was measured and it also allows the user to see easily and more clearly the shape of the head.



The objectives of the first phase were achieved, with the development of the application with graphical interface able to treat and analyse the clouds of points, in order to obtain the points and reference measures.

Keywords

Positional Plagiocephaly, Automatic Measures, Point Clouds

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