CRIB Annual Meeting

16 January 2020

2D67, Frenchay Campus

UWE Bristol | University of the West of England

Centre for Research in Biosciences Annual Meeting
16th January 2020
2D67

PROGRAMME

08:30 - 09:15 **Registration**

09:15 – 09:25 **Welcome**
Professor Aniko Varadi, CRIB Director

09:25 – 10.00 **Professor Gary Foster,** University of Bristol
Everything is connected; pathology, terrorism and drugs.

Theme 1 **PLANT STRESS AND DISEASE**
Chairs: **Heather Macdonald & Megan Richardson**

10:00-10:15 **Heather Macdonald**
Which way is up? Shedding light on new roles for ABA in algae, plants and maybe humans too.

10:15-10:30 **Megan Richardson**
Rapid screening for Cacao Swollen Shoot Virus also provides a tool for safe germplasm movement.

10:30-10:45 **Helen Neale**
Unbiased detection of *Pseudomonas syringae* genes regulating colonisation and persistence in Cherry canker.

10:45-11:10 **Poster viewing and Refreshments**

Theme 2 **HUMAN CHRONIC DISEASES & BIO-SENSING AND DIAGNOSTICS**
Chairs: **Alexander Greenhough & Elisabeth Slade**

11:10-10:25 **Alexander Greenhough**
Understanding hypoxia-induced signalling mechanisms during microenvironment-driven cancer cell adaptation.

10:25-11:40 **Elisabeth Slade**
Differentiation of wound associated bacterial biofilms using selected ion flow tube mass spectrometry (SIFT-MS) for the detection of volatile metabolites.

11:40-11:55 **Jason Mansell**
Out of the blue - Mussel-inspired platforms for bone biomaterial functionalisations.

11:55-12:30 **STUDENT FLASH POSTERS**
Chair: **Farnon Ellwood**

4 slides, 4 mins

1. **Amy Smart**
2. **Harshini Asurappulige**
3. **Jerro Saidykhan**
4. **Matteo Fois**
5. **Terry Devine**
6. **Liana Gynn**

12:30-13:00 **Lunch break**

13:00-13:30 **Poster Viewing**

Theme 3 **ENVIRONMENTAL CHALLENGES**
Chairs: **Gillian Clayton, Pedro Lafargue & Ross Bramston**

13:30-13:45 **Gillian Clayton**
Airborne microplastics; an unquantified risk.

13:45-14.00 **Pedro Lafargue**
The use of biomarkers as a tool to predict the origin of sustainable cacao beans to improve the chocolate supply chain.

14:00-14:15 **Ross Bramston**
Chemical free, mineral water swimming pool filtration system with biological treatment.

14:15-14:30 **Eva Perrin**
Assessing Ecological Health: The use of in-situ fluorescence sensing for monitoring biological processes in aquatic systems.

14:30-14:45 **Joshua Steven**
The control of waterborne pathogens using biofiltration.

14:45-15:00 **Farnon Ellwood**
Bird's nest ferns promote resource sharing by centipedes.

15:00-15:20 **Poster viewing and Refreshments**

Theme 4 **CANCER, GENETICS AND GENOMICS**
Chairs: **Ruth Morse & James Robson**

15:20-15:35 **James Robson**
The contribution of gut bacteria to colorectal tumour progression.

15:35-15:50	**Hartwig Visser** Differential DNA damage response kinetics direct cell fate.
15:50-16:05	**Ruth Morse** Is vaping really safer than cigarette smoking?
16:05-16:15	**Concluding remarks** **Dawn Arnold,** Deputy Director of CRIB

POSTERS

Alexandros Stratakos
Polyphenols from Brown Seaweeds as a Potential Antimicrobial Agent in Animal feeds.

Amy Smart
Voltammetric analysis of vitamin B1 using cobalt phthalocyanine screen printed carbon electrodes.

Molly Gillett and Matt Carter
The development of an optimised high resolution melt (HRM) method for the analysis of the efficacy of azacytidine as a hypomethylating agent.

Harshini Asurappulige
Chemotherapy-induced cytokine expression in a model of the human bone marrow.

Jerro Saidykhan
A printed paper-based device for measuring fibrinogen in resource-scarce and emergency settings.

Kevin Honeychurch
Anodic Stripping Voltammetric Determination of Zinc at a 3-D Printed Carbon Nanofiber Graphite Polystyrene Electrode.

Matteo Fois
Dual modality sensors for the detection of volatile organic compounds.

Terry Devine
Can volatile organic compounds from urine be used in the detection and ongoing management of bladder cancer.

Liana Gynn
Influence of Bone Marrow Stromal and Leukemic Cells on Cytarabine Chemo-Toxicity in Acute Myeloid Leukemia (AML)

Oral Presentations Abstracts

Which way is up? Shedding light on new roles for ABA in algae, plants and maybe humans too.

Layla Al-Hijab, Heather Macdonald, Michael Ladomery, Ian Wilson

CRIB, UWE Bristol

The phytohormone abscisic acid (ABA) is best known for its role in controlling water status in plants during the development of seed dormancy and under various stress conditions. More recently it has been shown to play roles in stress signalling in many other organisms, from green algae to humans. We investigated its role in the flagellated unicellular green alga Chlamydomonas reinhardtii. In the dark, ABA causes the algal cells to swim upwards and this effect is more marked when the algae are sampled towards the end of the night period. They also swim horizontally towards a fixed ABA source in the light, but away in the dark. This interaction of light and ABA signalling helps algae to position themselves optimally in the water column to minimise photo-oxidative stress and optimise photosynthesis. We hypothesise this was important in the evolution of land plants and suggest it could be key in controlling directional growth of cells in plants and perhaps other organisms.

Rapid screening for Cacao Swollen Shoot Virus also provides a tool for safe germplasm movement.

Megan Richardson[1] Andy Wetten[1] Joel Allainguillaume[1]

CRIB, UWE Bristol

Cacao Swollen Shoot Virus (CSSV) is the most destructive viral pathogen to have ever affected the West African cocoa crop. Symptoms of Cocoa Swollen Shoot disease on the Theobroma cacao host plant include red-vein banding along young leaves, cacao pod shape distortion and stem swellings. To date, the most effective method of CSSV control is the removal of symptomatic T. cacao and surrounding trees, though this has at best merely slowed the spread of the disease. While PCR-based detection of CSSV is possible using DNA extracted from cocoa leaf tissues this approach is time consuming, expensive and can lead to false negative results. In this work qPCR has been used to establish the effectiveness of a rapid CSSV screening approach based on peeled stem sections that do not need to undergo DNA extraction. Stem samples from CSSV-infected T. cacao were peeled at the vascular cambium and submerged for two hours in water from which CSSV was successfully detected. This study demonstrates the need for appropriate primer design and the precautions required to eliminate PCR inhibitors from the tissue exudates. The results help explain the disease pathogenesis, shedding light on the tissue-dependent distribution of the virus and also have implications for the international exchange of cocoa germplasm necessary for the improvement of the crop.

Unbiased detection of Pseudomonas syringae genes regulating colonisation and persistence in Cherry canker.

Helen Neale[1] MT Hulin[2] RJ Harrison[2] RJ Jackson[3] DL Arnold[1]

[1]CRIB, UWE Bristol
[2]Genetics, Genomics & Breeding Department, NIAB-EMR, Kent.
[3]School of Biological Sciences, University of Reading, Reading.

Pseudomonas syringae is a bacterial pathogen of many important crop species. The species is divided into more than 50 pathovars (pv) which are specialised to particular plant hosts. A subset of pathovars are pathogens of woody plants and cause canker in hosts including stone fruit, kiwi, hazelnut and horse chestnut. The disease is currently an unmanageable problem in stone fruit crops of the genus Prunus, which includes economically important species such as cherry, plum and apricot. The disease is an annual issue for most of the industry, in particular young orchards can be devastated, with tree losses sometimes reaching 75%. A lack of fully resistant varieties and effective control measures has hindered progress in combating this disease. Bacterial canker of Prunus is caused by at least three distinct clades of P. syringae, these are P. syringae pv. syringae (Pss), P. syringae pv. morsprunorum (Psm) race 1 and 2. This project aims to identify genes that are important in colonisation and pathogenicity. In order to do this I created transposon (Tn) mutant libraries in one strain from each of the three clades, Pss 9644, Psm MH001 and Psm 5244. These mutant libraries were subjected to several phenotypic assays including pathogenicity in Cherry fruit, colony size, biofilm formation, growth in vitro and swarming ability. These screens represent proxies of in planta growth traits and allow us to identify mutants with altered phenotypes. In total 778 mutants out of 3000 showed a significant change in one or more of these traits compared to the wild type strain. Of these mutants, 12 that showed complete loss of pathogenicity and 8 that were hyper-virulent were selected. The targeted genes were identified and the mutations complemented. Among the genes were several known to be involved in pathogenicity including Type III effectors and Flagella genes and also several previously unknown genes that may be important in bacteria-plant interaction and could be potential targets for disease control.

Understanding hypoxia-induced signalling mechanisms during microenvironment-driven cancer cell adaptation

Alexander Greenhough[1] Dr Kate J Heesom[2] Dr David B Gurevich[2] Dr Karim Malik[2] Dr Wes Kroeze[3] Prof Paul Martin[2] Prof Owen J Sansom[4] Prof Ann C Williams[2]

[1] CRIB, UWE Bristol
[2]University of Bristol
[3]University of North Carolina at Chapel Hill, US
[4]Cancer Research UK Beatson Institute, Glasgow

Oxygen depletion (hypoxia) in tissues occurs in many pathophysiological settings that feature an impaired blood supply, including wound healing, inflammation, obesity and diabetes. Hypoxia is also a hallmark of the tumour 'microenvironment' that drives malignant progression, spread and resistance to therapies. Regions of hypoxia are a common feature of bowel and pancreatic cancers — diseases for which new therapies are urgently needed. Although targeting hypoxia for cancer therapy has attracted much attention in recent years, identifying tractable molecular targets that can exploit the tumour-specific nature of hypoxia remains a major challenge.

Using proteomics and genetic loss of function approaches, I recently identified a new hypoxia-induced G protein-coupled receptor (GPCR) that enables cancer cell survival during oxygen deprivation. This GPCR is an 'orphan' receptor of poorly understood function: my hypothesis is that this receptor is a key microenvironmental 'sensor' that connects the extracellular milieu to adaptive intracellular responses.

With recently awarded funding from the Wellcome Trust, Bowel Cancer UK, and a Vice-Chancellor's ECR Award, I am beginning to establish a programme of research centred on understanding hypoxia-induced signalling mechanisms during cancer development. I am using functional cell biology approaches in 2D/3D tissue culture models of intestinal and pancreatic tumours, combined with proteomics, transcriptomics and bioinformatics. The findings are expected to provide fresh insights into the biological consequences of hypoxia, with relevance to physiological as well as pathophysiological settings. The outcomes and resources resulting from this project will inform future investigations into new molecular targets to potentially exploit disease-specific hypoxia for therapy.

Ultimately, since hypoxia is a key physiological hallmark distinguishing cancers from healthy tissues, understanding mechanisms of hypoxic cancer cell survival will enable the discovery of drugs that selectively kill cancer cells and spare normal tissues.

Differentiation of wound associated bacterial biofilms using selected ion flow tube mass spectrometry (SIFT-MS) for the detection of volatile metabolites

Elisabeth Slade[1] Robin Thorn[1] Amber Young[2] Darren Reynolds[1]

[1] CRIB, UWE Bristol
[2]Scar Free Foundation Centre for Children's Burns Research, Bristol Royal Hospital for Children, Bristol

Rapid, non-invasive diagnosis of wound infection would improve patient outcomes, as well as facilitate appropriate prescribing of antibiotics and reduce over-use. Microorganisms produce a wide range of volatile compounds (VCs) as a result of normal metabolism. Our previous research has shown that bacterial volatile profiles can be used to differentiate between species when grown in planktonic culture. The main aim of this study was to determine whether this approach could be used to discriminate between three species of pathogenic bacteria associated with wound infection, when grown in a collagen wound biofilm model.
Three strains each of Staphylococcus aureus, Pseudomonas aeruginosa and Streptococcus pyogenes were grown as biofilms using a collagen based growth matrix perfused with simulated wound fluid. Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) in full scan (FS) mode and selected ion mode (SIM) was used to analyse biofilm headspace gases, and multivariate statistical analysis employed to determine if volatile profiles could be utilised to discriminate between species.
Biofilms were analysed using SIFT-MS in FS and SIM modes, and volatile data analysed using multivariate analysis of variance to select potentially discriminant product ion peaks or VCs. The resultant data sets were analysed using hierarchical cluster and principal component analysis, visualised by constructing dendrograms and scatter plots of principal component scores. This approach resulted in species specific clustering of microbial biofilms using both FS and SIM data.
This study has demonstrated that steady-state biofilms of S. aureus, P. aeruginosa and S. pyogenes, can be differentiated based on analysis of headspace gases. We have shown that through utilising SIFT-MS, volatile product ions and specific VCs can be used to discriminate between bacterial species associated with wound infection based on analysis of headspace gases of continuous culture biofilms. This work lays the foundations for future development of a non-invasive diagnostic tool for rapidly diagnosing wound infection.

Out of the blue - Mussel-inspired platforms for bone biomaterial functionalisations.

Jason Mansell[1] Fiona Baldwin[1] Anna I. Shiel[1] Tim J. Craig[1]

[1] CRIB, UWE Bristol

Osteoarthritis is a common crippling affliction with devastating consequences for synovial joints. Advanced disease often necessitates removal of the affected joint and replacement with a prosthesis, often made from titanium (Ti). Whilst survival of implants is good, around 7% will fail through a process known as aseptic loosening. The socioeconomic impact is significant, indeed the cost incurred for replacing failed devices is in excess of £300m per annum in England and Wales alone. There is a clear incentive therefore to find ways of enhancing implant longevity. One potential solution is to coat the implant surface with biological agents that target the bone forming osteoblast cells. To this end we have taken inspiration from the growing interest in polydopamine (PDA) capturing platforms following reports that PDA interacts with lipids and phosphonic acids. The emergence of PDA in (bio)material design is based on catechol chemistry of the adhesive proteins used by edible Mussels (Mytilus edulis) in securing their attachment to wet surfaces. We have adopted a "one-pot" Ti-coating strategy wherein samples are steeped in a mildly alkaline solution of dopamine spiked with the lysophospholipid (3S)1-fluoro-3-hydroxy-4-(oleoyloxy)butyl-1-phosphonate (FHBP). We chose FHBP following our discovery that it co-operates synergistically with vitamin D3 to promote human osteoblast maturation. Compared to blank Ti, FHBP-Ti and PDA-Ti, we found the hybrid FHBP-PDA-Ti surfaces far superior in supporting human osteoblast maturation. Since osteoblast maturation is synonymous with the provision of a mechanically robust bone matrix the type of coating we are developing has the potential in delivering devices with enhanced longevity.

Airborne microplastics; an unquantified risk

G Clayton[1] S. Sargeant[1] B. Williams[2] K. Lamb-Riddell[3] T. Cox[3] L. Mol[4] D. Reynolds[1] E. Hayes[2]

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Microplastics [MPs] are particles or fibres that are less than 5 mm in size, and are generally divided into primary and secondary MPs. Primary MPs are commonly derived from personal care and medical products, whilst secondary MPs are degraded fragments or fibres. In recent years, MPs have become ubiquitous in nature, with many studies reporting the presence of MPs in aquatic, terrestrial and, more recently, in atmospheric systems. The aim of this study was to develop a standard protocol for the recovery, identification, characterisation and quantification of microplastics present in air.

A sample preparation and recovery method was developed using five model reference plastics: polyamide 6 (nylon) fibre, polypropylene granules, regenerated cellulose (rayon) fibres, high-density polyethylene granules and polyester fibres. The sample preparation method was then applied to β-attenuation monitoring [BAM] filters supplied by an English Local Authority. Briefly, samples were suspended in 20 mL 35% laboratory grade ethanol. Samples were then sonicated for 5 minutes, and vortexed for 30 seconds, twice in total before undergoing acid digestion. Samples were then filtered through 0.2µm filters, within a sterile glass vacuum filtration unit. Filters were then placed on 70mm filter paper in petri dishes, and left to dry over night at 37°C ± 1°C. Samples were then weighed to quantify non-organic particles present in samples, before being characterised through micro-Raman spectroscopy.

The mean percentage recovery for reference model plastics was determined using the developed sample preparation method. Preliminary results confirmed MPs > 2.5 µm were present on BAM filter samples using the developed sample preparation method. Future work will continue to refine the sample preparation methods as well as quantify, identify and characterise MPs present from environmental samples. BAM automatic samplers are widely used to monitor air quality for statutory purposes across the UK. Our protocol provides a mechanism to assess and quantify airborne MP concentrations at a national scale.

The use of biomarkers as a tool to predict the origin of sustainable cacao beans to improve the chocolate supply chain.

Pedro Lafargue , Joel Allainguillaume

CRIB, UWE Bristol

Supply chain and stakeholders analysis:

To identify the factors that influence cacao traceability and the importance of assessing it in different supply chain systems from small-scale farmers to corporations, various stakeholders and processes have been mapped by principal activity. Information from interviews and questionnaires showed that stakeholders from a wide range are willing to implement various technologies in order to decrease the cost, optimize and improve the tracking systems of cacao and chocolate products.

Chloroplast plant DNA Markers and data modelling:

Chloroplast ultra-barcoding in cacao has revealed a level of DNA polymorphism sufficient to reliably identify lineages below the species level such as subspecies or varieties.

All accessions were screened with 25 (cpSSR) and Indel markers to assess the overall chloroplast genetic diversity of the crop. The same loci were screened on chocolate samples and proportion of alleles at each locus calculated. Chloroplast haplotypes were generated from the allelic data from the reference samples providing a complete cover for all haplotypes present in the crop. I designed a new model based on cross sectional regressions to detect the proportion of these haplotypes in DNA samples obtained from chocolate. The model uses the allelic proportion data generated by capillary analysis and the average proportion of each haplotype group calculated from the reference samples. For example, a random chocolate sample was screened and the data analysed through the model using 20 reference haplotypes. The composition of haplotypes was predicted as follow: haplotype A (46.5%), haplotype B (17.3%), and haplotype C (17.3%) with 18.9% from other haplotypes. The three combined haplotypes explain 84% of the variability of the chocolate sample. This approach to identify proportion and provenance of chocolate products can be implemented not only with chloroplast markers but with any data generated from biological markers including plant or microbiome genome, isotopes, metabolites or volatile components.

Chemical free, mineral water swimming pool filtration system with biological treatment.

Ross Bramston[1] Joshua Steven[2]

[1]KTP Associate: Hydrolize Ltd and UWE Bristol
[2]CRIB, UWE Bristol

Swimming pools filtration systems typically use some form of chemical means for disinfecting pool water. They are resource intensive and costly to run. Hydrolize ltd, in partnership with faculties Health and Applied Science (HAS) and Engineering Technology (FET) at UWE, is in the process of developing a chemical free swimming pool filtration system that integrates biological filtration with mechanical filtration.

Biological filtration systems offer a vast reduction in energy consumption than competitor systems and mitigate the need for chemical additives. As a development to Natural Swimming Pools (NSPs), the technology pioneered by Clear Water Revival has been adapted to an outboard system that can compete with typical filtration systems. NSPs have previously been bespoke builds and more costly to construct than traditional swimming pools, the Hydrolize Filtration system can be produced in greater volume, at a reduced cost and can be installed on new builds or retrofitted to existing swimming pools, both indoor and outdoor. Minimal on-site installation is needed, maintenance no longer requires handling harmful chemicals and users benefit from swimming in chemical free mineral water.

Operating at atmospheric conditions, there is little in the need of input. The system employs natural means to break down ammonia into usable products for the biofilter, thus removing organic material from the water column whilst improving both the purity and clarity of the water.

In addition, there is potential for adaptation to a range of projects of critical importance, from river remediation to creating drinking water from polluted sources.

Assessing Ecological Health: The use of in-situ fluorescence sensing for monitoring biological processes in aquatic systems.

Eva Perrin[1] Darren Reynolds[1] Robin Thorn[1] Stephanie Sargeant[1]

[1]CRIB, UWE Bristol

Global and regional change will heavily influence the structure and function of aquatic ecosystems around the world. Globally, freshwater systems are under increasing pressure from anthropogenic perturbations, with land-use and agriculture exerting enormous pressures on these environments, and compromising their ability to deliver ecosystem services. Whilst the EU Water Framework Directive defines ecological status as 'a measure of the quality of the structure and functioning of surface water ecosystems', conventional water quality parameters seldom incorporate process-based principles in their assessments of surface freshwater quality.

The aim of this project is to employ recently-developed, novel in-situ fluorescence sensing technology to develop our understanding of the sources, transport and fate of aquatic dissolved organic matter (DOM) within freshwater ecosystems. The purpose of this is to understand how microbial processes respond to changes in land-use within surrounding catchments, and observe how this influences the fluorescence characteristics of the freshwater DOM pool. The deployment of the novel fluorosensor for real-time, in-situ monitoring is being integrated in tandem with discrete sampling procedures and on-site knowledge of land-use changes and pollution events both in the UK and overseas. This will ultimately help to develop a suite of meaningful water quality information pertaining to fundamental ecosystem processes, which will give an insight into the underpinning ecosystem health and represent a valuable water quality parameter in its own right

The control of waterborne pathogens using biofiltration.

Joshua Steven[1] Robin Thorn[1] Gareth Robinson[1] Dann Turner[1] Darren Reynolds[1]

[1]CRIB, UWE Bristol

Biological filters are widely used throughout the wastewater sector for reducing inorganic contaminants and organic material. Even so, little is known regarding the use of biofilters for the control and/or removal of pathogenic bacteria in drinking water supplies. Currently, the consumption of water contaminated with pathogenic microorganisms causes between 1.7 and 2 million deaths each year from diarrhoeal diseases. The aim of this study was to investigate the control of pathogenic bacteria commonly associated with biologically contaminated water using biofiltration. In this study, laboratory-scale and upscaled test biofiltration systems were used to investigate the control of waterborne pathogens. Using the laboratory-scale biofilters, potable water was inoculated with Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa (starting range inocula of between 300 - 1000 CFU 100 mL-1) to challenge the test biofilters. With respect to the initial pathogen load, the matured biofilter was shown to significantly reduce E. faecalis (67% Â± 14%), E. coli (58% Â± 22%) and P. aeruginosa (76% Â± 4%) after a series of 4 filter passes, equating to a total contact time of 8 min and 24 secs. For the upscaled biofilters, environmental water contaminated with waterborne pathogens including E. coli and faecal coliform bacteria (starting density range of between 10 - 200 CFU 100 mL-1) was used to challenge the test biofilter system. With respect to the starting density, the test biofilter system reduced E. coli (79% Â± 20%) and coliform bacteria (84% Â± 23%) after 2 hours of operation at a flow rate of 3.6 m3 h-1. Overall, this work demonstrates that biofilter systems can be successfully used to reduce E. faecalis, E. coli, P. aeruginosa and coliforms from a source water. Further research is required to understand the mechanisms by which biofilters reduce the number of viable pathogens in a source water.

Bird's nest ferns promote resource sharing by centipedes.

Farnon Ellwood[1] Josie Phillips[1] Arthur Chung[2] Gregory Edgecombe[3]

[1]Centre for Research in Biosciences, University of the West of England, Bristol, UK
[2]Sabah Forestry Department, Forest Research Centre, Sandakan, Malaysia
[3]Department of Earth Sciences, The Natural History Museum, London, UK

Bird's nest ferns (Asplenium spp.) support large numbers of invertebrates, including centipedes. As top invertebrate predators, centipedes drive ecosystem function, for example, by regulating decomposer populations, but we know little of their ecology in forest canopies. We provide the first detailed observations of the diversity and structure of the centipede communities of bird's nest ferns, revealing the importance of these epiphytes as nurseries for centipedes. We collected 305 centipedes equating to Ëœ11,300 mg of centipede biomass from 44 bird's nest ferns (22 of which were from the high canopy and 22 from the low canopy) in primary tropical rainforest in Sabah, Malaysian Borneo. Most abundant were the Scolopendromorpha (n = 227 individuals), followed by the Geophilomorpha (n = 59), Lithobiomorpha (n = 14), and Scutigeromorpha (n = 5). Although we observed very little overlap in species between the forest strata, scolopendromorph centipedes dominated throughout the canopy. Null model analysis revealed no significant competitive interactions; on the contrary, we observed centipedes sharing nest sites within the ferns on three of the ten occasions that we found nests. All nests belonged to centipedes of the family Scolopendridae, which are typically aggressive, and usually show negative spatial association.

This study reveals a diverse community of canopy centipedes, providing further evidence of the importance of bird's nest ferns to a wide range of animals, many of which use the ferns at critical life stages. Future conservation strategies should regard these ubiquitous epiphytes as umbrella species and protect them accordingly in landscape management decisions.

The contribution of gut bacteria to colorectal tumour progression.

James Robson[1] ,Robin Thorn[1] ,David Qualtrough[1]

[1]CRIB, UWE Bristol

Colorectal cancer (CRC) is the second leading cause of cancer related death in the UK. Development occurs over many decades, and is heavily influenced by environmental factors, including the gut microbiome. Some bacteria are enriched in cancer patients, and have been linked to carcinogenesis. It remains unclear, however, whether these bacteria play a prominent role in cancer progression, or are simply better adapted to the tumour microenvironment than other gut residents. The aim of this research was to determine whether bacterial stimulation can drive the advance from benign tumour to malignant cancer.

The effects of bacteria on the benign colorectal adenoma cell line RG/C2 and the malignant colorectal carcinoma cell line HCT116 were compared. Differences in the interactions of gut bacteria with benign and malignant tumour cells were analysed using the gentamicin protection assay. Tumour cells were treated with bacterial metabolites or directly with bacterial cells in vitro, and the ability of bacterial stimuli to drive tumour growth and motility were assessed.

All bacteria studied were able to attach to and invade benign and malignant tumour cells. After bacterial treatment, a dramatic increase in cell growth was observed. The fold change in cell growth was equivalent for both cell lines. Similarly, an increase in cell yield was observed when cells were treated with conditioned medium from some species. Transwell filter migration assays demonstrated that two species, Escherichia coli Nissle 1917 and F. nucleatum, were able to drive cell motility in HCT116 cells, but not in the RG/C2 cell line; this was then confirmed via the wound healing assay.

These results indicate that gut microbiome constituents may influence colorectal tumour progression via different mechanisms, depending on the disease state. Bacterial stimulation was able to drive tumour cell growth in benign and malignant cells, but was not able to confer a migratory phenotype in pre-malignant tumour cells. Future work will aim to explain the observed effects at the gene expression level.

Differential DNA damage response kinetics direct cell fate.

Hartwig Visser[1] Ruth Morse[1] Adam Thomas[1]

[1]Centre for Research in Biosciences, UWE Bristol

Cells are constantly exposed to DNA damage resulting from internal processes (e.g. cellular respiration), or external sources (e.g. ultraviolet radiation). DNA damage can also be induced therapeutically (e.g. cancer treatments). However, while naturally acquired damage does not usually result in cell death, cancer treatment often does. This suggests a dichotomous DNA damage response (DDR), able to distinguish from repairable (sub-lethal) and irreparable (lethal) damage. This forms the basis of our hypothesis as we aim to determine DDR dynamics at sub-lethal and lethal doses. Firstly, TK6 cells were exposed to a concentration range of temozolomide (TMZ) (chemotherapeutic drug) to identify a sub-lethal and a lethal dose. Functional analysis of apoptosis was performed by flow cytometry measuring Annexin-V over increasing doses of TMZ at 24 and 48 hours. Thirty micromolar and 900 μM TMZ were chosen based on their differential apoptotic index and were respectively considered sub-lethal and lethal at 24 hours. We measured γH2Ax foci, as a biomarker for DNA damage, in TK6 cells by immunofluorescence microscopy 24 h post-exposure to these doses of TMZ. At 30 μM TMZ, the levels of damage did not statistically differ from controls, whereas at 900 μM, a significantly ($p = 0.004$) greater proportion of cells displayed elevated γH2Ax foci. Subsequent analysis of activated (phosphorylated) checkpoint kinase 1 (Chk1), a key DDR protein reveals that 30 μM and 900 μM TMZ illicit a differential DDR. At 30 μM, little variation in phosphorylated Chk1 (pChk1) was observed compared to controls, whereas 900 μM showed pronounced elevation. These data suggest there is a differential DDR at sub-lethal and lethal doses that directs cell fate from potential repair and survival, to apoptosis.

Is vaping really safer than cigarette smoking?

Ruth Morse[1] Ryan Johnson[1] Abigail Parker-Oddy[1]

[1]CRIB, UWE Bristol

Health risks from tobacco particulate (TP) are well documented, but less is understood for e-cigarettes. E-cigarette misuse has led to fatality through nicotine overdose and deliberate ingestion, however recent reports are claiming e-cigarette related lung disease. There is conflicting data of e-cigarette toxicity, however most in vitro data is based on simple single cell cultures, often not utilising lung cells and not considering normal lung physiology. The normal lung contains copious macrophages which release reactive oxygen species during phagocytosis; these may induce toxicity in exposed lung.

Here, we observed smokers for time spent smoking, time between sessions and time between inhalations to estimate exposure to TP and e-cigarette fluid (vape). Additionally, TP and vape was trapped on filter paper, dissolved in DMSO, and A549 (lung epithelial) and U937 (macrophage from human lung) were separately and in co-culture exposed to TP and vape. Cells were observed for cytotoxicity and genotoxicity.

Whilst there was no significant difference in time between inhalations for either smoking or vaping, the time between smoking episodes was significantly shorter ($p<0.001$) for vaping, and smoking episodes were significantly longer for vaping ($p<0.01$). These data infer that exposure to vape may be higher than for TP.

Viability studies showed that macrophages alone were resistant to exposure (80.9% vs 71.9% vs 77.3%; untreated v TP v vape with S9), whereas lung cells succumbed to TP (47.46%) and lost adherence, and vape caused poor morphology but less cell death than TPM (69.7%, with S9). Intriguingly, macrophages altruistically protected lung cells in co-culture (lung viability 80.9% vs 63.3% vs 76.3%; unt vs TP vs vape). Genotoxicity in lung cells was also higher when in co-culture with macrophages. Our co-culture model demonstrates that simple assays may misrepresent toxicity, when normal physiology is not considered. Furthermore, whilst equivalent doses of vape do not appear as toxic as TP, the potential higher exposure in smokers should be taken into consideration.

Poster Presentation Abstracts

Poster 1

Alexandros Statakos

Polyphenols from Brown Seaweeds as a Potential Antimicrobial Agent in Animal Feeds.

Seaweeds offer a natural source of antimicrobials that may help curb antibiotic-resistance in livestock. The antibacterial activity of phlorotannin extracts isolated from two brown seaweeds Ascophyllum nodosum and Fucus serratus were tested. The mechanism of action of phlorotannin extracts against Escherichia coli O157, Salmonella agona and Streptococcus suis was elucidated by observing cell membrane permeability and intracellular ATP. The two extracts were effective at killing three foodborne pathogens without negatively affecting the pig intestinal cells. A. nodosum MIC range for the different pathogens was between 1.56 to 0.78 mg/mL, whereas F. serratus was 3.13 mg/mL for all pathogens tested. A. nodosum was found to be much more potent compared to F. serratus. The difference in potency in the seaweeds may be a result of the phlorotanninsâ€™ structural linkages. The antimicrobial properties of the seaweed extracts tested may provide alternative and complementary treatments to antibiotics and zinc oxide in animal feeds.

Poster 2

Amy Smart

Voltammetric analysis of vitamin B1 using cobalt phthalocyanine screen printed carbon electrodes

Vitamins are an important group of organic compounds which have a role in many biological processes. Vitamins are commonly found in foods (naturally or through fortification), pharmaceuticals and cosmetics. Vitamins possess electrochemical properties which have been studied using a range of electrode materials, including platinum, mercury and glassy carbon. Few studies have used screen printed carbon electrodes with redox mediators for vitamin analysis. There are many advantages of screen printed carbon electrodes, such as low cost and potential for mass production.

Cyclic voltammetry was used to ascertain the electrochemical behaviour of thiamine (vitamin B1) using cobalt phthalocyanine screen printed carbon electrodes vs Ag/AgCl. A pH study confirmed that vitamin B1 is not electrochemically active under neutral conditions, however under basic conditions it is converted to an electrochemically active thiol [1]. This derivative has a peak potential in the negative voltage window using the electrodes described. A scan rate study was performed to provide information concerning the nature of the electrode reaction at the electrode surface [2]. A calibration study was performed using the optimum solution conditions in conjunction with a suitable pulse voltammetric waveform. This poster will describe the performance characteristics of the resulting method.

References:

[1] Hart, J. P., Norman, M. and Tsang, S. (1995) Voltammetric behaviour of vitamin B1 (thiamine) at a glassy carbon electrode and its determination in multivitamin tablets using anion-exchange liquid chromatography with amperometric detection under basic conditions Analyst. 120, pp. 1059-1064.

[2] Westmacott, K. L., Crew, A., Doran, O. and Hart, J. P. (2018) A novel electroanalytical approach to the measurement of B vitamins in food supplements based on screen-printed carbon sensors. Talanta. 181, pp. 13-18.

Poster 3

Molly Gillett and Matt Carter

The development of an optimised high resolution melt (HRM) method for the analysis of the efficacy of azacytidine as a hypomethylating agent

Myelodysplasia (MDS) negatively affects the bone marrow resulting in the production of immature, non-functional blood cells. This negative effect on blood cell production can be caused by epigenetic mutations in genes such as TET2, resulting in DNA hypermethylation that can progress to acute myeloid leukaemia (AML). Azacytidine (AZA) is a DNA hypomethylating agent well known for its ability to reduce methylation of DNA and prevent transformation to AML. However, it is not 100% effective due to acquired resistance, for which there is currently no monitoring. Therefore, a method of analysis must be developed in order to determine patient suitability for sustained AZA prescription. Prevention of condition progression to AML is the long-term aim of this study as this will both enable an improved patient quality of life as well as an increased life expectancy.

The KG-1a cell line was used as a model of MDS. Cytotoxicity of AZA was assessed through measurement of %viability and quantitation of ATP as a measure of metabolic activity in order to optimise the dosage of AZA. High resolution melt (HRM) was used to analyse global DNA hypermethylation. In brief, extracted DNA was enzymatically digested using methyl-sensitive and -insensitive restriction endonucleases HPAII that cuts at unmethylated CpG islands, and MSPI that cuts at CpG islands regardless of methylation. HRM allowed for determination of DNA fragment length dependent on the level of enzymatic restriction, which is inversely proportional to methylation status. An electrophoresis gel comparison confirmed successful DNA restriction. Gene specific promoter Q-PCR of TET-2 was also investigated as a comparison to the global genetic HRM methylation assay. It is hoped this will have a significant impact on patient treatment timelines as valuable time will not be wasted on an unsuccessful treatment.

Poster 4

Harshini Asurappulige

Chemotherapy-induced cytokine expression in a model of the human bone marrow.

Chemotherapy induces a 'cytokine storma' in patients, usually peaking a few days after end of treatment causing multiple complications including cytotoxicity. Our group has shown that chemotherapy can induce a bystander effect (BE) producing cytotoxicity and genotoxicity, with peak induction of this BE aligning with the observed cytokine expression.
This research will explore the role of cytokine expression in inducing mutagenic events in transplanted donor stem cells, leading to donor cell leukaemia (DCL). Cytokines are highly polymorphic, which might explain why some patients get DCL and others donâ€™t. DCL largely presents as acute myeloid leukaemia, which may result from cytokines involved in myeloid lineage differentiation.

The HS-5 stromal cell line was exposed to clinical doses of mitoxantrone (MTX) and chlorambucil (CHL), then allowed to condition culture medium at days 2 and 3 resp., aligning with peak induction of BE for these drugs. Conditioned medium was harvested and tested for 80 cytokines using an array. The array was analysed for absolute and fold-changes in cytokine expression.

GM-CSF, IL-6, MCP-2 and TNFÎ± were the four highest cytokines expressed for both drugs, however other highly expressed targets included RANTES, TIMP-2, MIP-3a, MCP-1, MCP-3, MCP-4, GCP-2, NAP-2, GRO-Î±, osteoprotegerin, IL-8 and IL-1Î². GM-CSF and IL-6 are involved in myeloid differentiation. However, these cytokines are highly expressed in untreated cells, so fold-change was performed. SCF was the most notable fold-increase (~25x and ~60x higher for CHL and MTX resp.); also important in myeloid differentiation. A further 26 cytokines were upregulated by both drugs, including RANTES and TNFÎ±.
Further work will explore these highlighted candidates for the ability to induce cytotoxic and genotoxic endpoints in bystander cells. Primary samples will be genotyped for polymorphic variants, and evaluated for potency in inducing a BE. These data may prove useful in identifying individuals at risk for chemotherapy complications, and may influence treatment strategies for individuals.

Poster 5

Jerro Saidykhan

A printed paper-based device for measuring fibrinogen in resource-scarce and emergency settings

Coagulation disorders cause excessive blood-loss, resulting in extensive morbidity and mortality, particularly in resource-poor settings. Death rates due to, e.g. haemorrhage during childbirth, venous thromboembolism, disseminated intravascular coagulation are high in certain countries/regions, particularly Africa. A lack of suitable blood coagulation tests prevents rapid diagnosis and treatment. A particular clinical challenge in low resource environments is post-partum haemorrhage (PPH), which is the cause of one quarter of global maternal deaths. One woman dies every seven minutes from PPH [1].

During pregnancy, the levels of clotting factors become elevated as a natural response to the increased risk of feto-maternal haemorrhage. However, the severity of PPH can increase significantly particularly if fibrinogen levels are low. Thus, fibrinogen is an important marker of severe haemorrhage. Rapid measurement before, during, and following parturition would allow more effective intervention, with the potential to reduce mortality and morbidity.

While a number of devices have been developed for point of care measurement of coagulation [2-3], including fibrinogen [4], these are either too technologically complex for low-resource settings, or are based on fabrication methods and materials that are not sustainable or supportive of current environmental challenges, particularly relating to the use of plastics [5]. Paper-based bio-devices are growing significantly as an area of research and technological development due to their potential for low cost production, ease of use and resource sustainability [6].

A device for measuring blood plasma fibrinogen has been developed, which is based on wax-printed chromatographic paper strips modified with ink-jet printed thrombin reagent. Clotting of the blood plasma sample coming in contact with the thrombin reagent is induced and the flow rate of the sample is proportional to the fibrinogen concentration, which can be measured in the 0 to 7 g/L range.

Poster 6

Kevin Honeychurch

Anodic Stripping Voltammetric Determination of Zinc at a 3-D Printed Carbon Nanofiber-Graphite-Polystyrene Electrode

The application of a novel fully 3-D printed carbon nanofiber-graphite-polystyrene electrode has been investigated for the trace determination of Zn(II) by differential pulse anodic stripping voltammetry. The possibility of utilising a carbon pseudo-reference electrode was found to be successful. The effect of accumulation potential and time were investigated and optimised. Using an accumulation potential of -2.9 V (vs. C) and an accumulation time of 75 s a single sharp anodic stripping peak was recorded exhibiting a linear response from 12.7 Âµg/L to 450 Âµg/L. The theoretical detection limit (3Ï*f*) was calculated as 8.6 Âµg/L. Using the optimised conditions a mean recovery of 97.8 %, (%CV = 2.0 %, n = 5) for a tap water sample fortified at 0.990 Âµg/mL was obtained indicating the method holds promise for the determination of Zn(II) in such samples.

Poster 7

Matteo Fois

Dual modality sensors for the detection of volatile organic compounds

Volatile organic compounds (VOCs) have become a topic of great interest in several areas, and of great importance is the development of systems and devices able to detect unambiguously trace quantities of these compounds. For this purpose, in the last decades have been proposed, realized and tested a large number of sensor systems able to give a response to a diverse range of target (ranging from metabolites in human breath to explosives traces) In this context it has been possible to use different materials such as metal oxides largely based on their changes in the electrical resistance following the interaction with volatiles. Nevertheless, often these sensors present inadequate sensitivity and, especially in real life applications, a limited selectivity. A new frontier on this issue is to combine different signals produced from the same sensor – 'multimodal sensors'.

Recently, the University of the West of England patented and tested a new kind of rare earth doped metal oxide sensor system and this new technology is able to sense organic compounds with a dual modality response, combining a resistance and a cataluminescence response in order to achieve better selectivity towards target compounds.

The tests have been performed on a broad range of volatile targets and the sensors tested showed a high sensitivity (under the ppm limit), especially for acetone and ethanol. Furthermore, combining the different responses, it has been possible to increase the selectivity compared to existing sensor systems (de Lacy Costello B. (2018) Patent W&R Ref. P123791GB).

Poster 8

Terry Devine

Can volatile organic compounds from urine be used in the detection and ongoing management of bladder cancer

Bladder cancer is a problematic public health issue affecting thousands (549,000 p/year) of people throughout the world (Bray et al., 2018). Alarmingly, there are no screening programmes to assist in the early diagnosis of bladder cancer and subsequent treatment. If bladder cancer is suspected, diagnosis and management is typically painful whereby patients characteristically endure invasive and expensive examinations and follow-up procedures such as flexible cystoscopy. Hence, the urgent requirement for a cheap, rapid and non-invasive screening programme to detect bladder cancer during early stages and to inhibit post-operative procedures.

Urine samples were obtained from 58 patients, 29 with bladder cancer and 29 clinically relevant age matched controls. 1mL of urine from all samples was transferred into glass headspace vials, pre-treated with 1mL of 1M sodium hydroxide, and frozen at -80Â°C. The samples were defrosted at ambient room temperature for 15 mins, heated at 40Â°C for 15 mins, then purged with nitrogen for 2 mins at 80mL/min onto Tenax thermal desorption tubes ready for analysis using a novel ATD-GC-MS-Sensor.

GC-MS data was analysed using XCMS bioinformatics software to search for significantly different masses between the sample sets (cancer and non-cancer). Compounds were tentatively matched against a spectral database (NIST library). Of the 100+ compounds initially identified, only those compounds with p values less than <0.05 were selected and post analysed for identification. Eight significant compounds (Dichloromethane; Ethanol; 5-hepten-2-ol, 6-methyl; Trichloromethane; 2, 6-dimethyl, 2, 4, 6-octatriene; cis-Ocimene; Benzene-1-methyl-3-(1-methylethenyl); Limonene) were identified for bladder cancer discrimination. One of which, ethanol, was found to be raised in the cancer group and not in the control group.

Aside from contributing toward bladder cancer diagnosis and management, the method could be employed to similar disease identification fields commonly recognised for their invasive diagnostic approach such as prostate cancer detection.

Poster 9

Liana Gynn

Influence of Bone Marrow Stromal and Leukemic Cells on Cytarabine Chemo-Toxicity in Acute Myeloid Leukemia (AML).

Mesenchymal stromal cells (MSC) are known to protect leukemic cells from drug-induced toxicity in the bone marrow (BM), however less is known about the impact of co-culture on supportive MSC. Over a third of AML patients do not show continued response to cytarabine (Ara-C), with many resistance mechanisms still unknown. The DNA damage caused can persist in MSC and may be implicated in BM failure and secondary malignancies.

This study investigated the influence of MSC and leukaemic cells on chemo-toxicity; genotoxicity and cytotoxicity protection/sensitisation and the underpinning mechanisms.

Primary MSC, stromal (HS-5) and AML (HL-60, K562) cell lines were co-cultured, prior to treatment with Ara-C. Cytotoxicity was monitored by viability, proliferation and chemo-sensitivity assays, while genotoxicity was determined by micronucleus and alkaline comet assays. Differential cytokine secretion utilised an array, with quantification by ELISA.

In co-culture, stromal cells were sensitised to drug-induced cytotoxicity, while leukemic cells were themselves protected from treatment. Genotoxicity was also significantly increased in HS-5 and MSC, while being significantly decreased in leukemic cells when co-cultured, conferring with cytotoxicity findings. Similarly, BM-MSC from previously treated patients had significantly higher genotoxicity than patients at diagnosis. Seven of 36 cytokines were differentially secreted by cell lines in co-culture. Of these, the inflammatory cytokine, macrophage migration inhibitory factor (MIF), was decreased in co-culture, and has been implicated in the progression of other malignancies.

This study shows for the first time that the co-culture of AML and MSC alters the genotoxic effect of chemotherapy. Future research utilising larger patient cohorts is required to fully understand how cells in the BM can be targeted. This could potentially improve long-term complications of current therapies.