Conference report

Chemistry, Stem Cells and Regenerative Medicine

11 Sep 2017

RSC Chemistry Centre, Burlington House

This was the 2nd of a series on the subject of stem cells, following on from a highly successful conference in 2015.

The programme consisted of 8 speakers: 6 from the UK plus 1 each from Sweden and Norway. A total of 44 delegates attended, mostly from academia. Presentations were focused on how to determine the chemical and biochemical signals that control the fate of stem cells during normal healthy development and how this knowledge could be exploited to develop effective cell-based and non-cell-based therapeutics for tissue repair and regenerative medicine. Research studies demonstrated how medicinal and materials chemistry, particularly tissue engineering, are both playing a major role in this quest. There were 5 poster presentations: Jordi Gonzalez-Molina (UCL); Halacheva Silviya (University of Bolton); Kristine Laws (KCL); Francis Man (KCL); Neelima Thottappillili (University of Edinburgh).

Questions were addressed to the speakers at the end of their presentations resulting in good interactive discussions which continued during the breaks, particularly during the lunch time poster session. Feedback from the speakers and students was very positive and they endorsed the idea of holding a similar symposium every two years to parallel the progress being made in this exciting field.

The Names of the Speakers and the Titles of their presentations and also those of the poster presenters (+ their Abstracts) are in the Full Programme which can be found appended hereto.

The organising committee thank the RSC Chemistry Centre, Burlington House for their hospitality and assistance.

The organisers were:

Dr Irene Francois (Chair)

Dr Ivan Wall

Dr Francis Lister

Programme

09.30 Coffee and registration

10.00 SESSION 1
Chair: Doctor Irene François

10.05 Professor Mark Bradley (School of Chemistry, University of Edinburgh UK)
Polymers for the control & manipulation of stem cells

10.40 Doctor Gareth Sullivan (Norwegian Stem Cell Centre, University of Oslo, Norway)
Modulating stem cell fate - a farewell to growth factors

11.15 Professor Jöns Hilborn (Department of Chemistry, University of Uppsala, Sweden)
Engineering RNAi to regulate gene expression in vivo

11.50 Lunch and Poster Session

13.30 SESSION 2
Chair: Doctor Ivan Wall

13.35 Professor Liam Grover (School of Chemical Engineering, University of Birmingham, UK)
Designing materials to optimise regeneration

14.10 Professor Iain Gibson (Institute of Medical Sciences, University of Aberdeen, UK)
Using physicochemical cues to guide stem cell behaviour

14.45 Doctor Kogularamanan Suntharalingam (Department of Chemistry, King's College London, UK)
Taking the cancer stem cell gamble with cytotoxic metal complexes

15.20 TEA

16. 05 SESSION 3
Chair: Doctor Francis Lister

16.10 Doctor Heiko Wurdak (Leeds Institute of Molecular Medicine, University of Leeds, UK)
Investigating molecular vulnerabilites in high grade brain tumours using small molecules

16.45 Doctor Damian Marshall (Cell & Gene Therapy Catapult, London, UK)
The role for inferential control in reactive cell therapy manufacture

17.20 Closing Remarks

Poster Abstracts

Extracellular viscosity enhances mesenchymal migration and alignment in liver cancer cells

Jordi Gonzalez-Molina[1], Michela Borghesan[2], Barry Fuller[3], Clare Selden[1]

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The physicochemical properties of the microenvironment of cells greatly impact on their behaviour. Several pathological conditions affect the viscosity of the extracellular fluid including mucin-producing adenocarcinomas. Also, the use soluble polymers for cell transplantation may locally increase the extracellular viscosity. The aim of this study was to investigate the effect of inert soluble polymers, typically used in cell transplantation and as scaffolds for tissue engineering, on liver cancer cell behaviour.

First, a wound-healing assay showed that epithelial and mesenchymal cells responded differently to Na-alginate, with an inhibitory effect on the former and stimulatory on the latter. Interestingly, as observed in hydrodynamic shear stress studies with endothelial cells, SK-HEP-1 cells, of endothelial origin, aligned when cultured in viscous 1% Na-alginate (~200 cP), presenting a multicellular actin cytoskeleton alignment with significantly higher fibre anisotropy ($n = 3$, $p<0.001$, Student's t-test). Also, in SK-HEP-1 cells there was an average increase of 83% in cell area and a 46% reduction in shape factor ($4\square\square x$ Area/Perimeter^2) ($n=250$ cells, $p<0.001$, Student's t-test). Moreover, 1% Na-alginate enhanced the area of epithelial PLC/PRF/5 cells in a substrate stiffness-dependent manner. This effect was accompanied by a higher nuclear localisation of the rheostat protein YAP only in cells adhered to fibrotic liver-like stiffness substrates (10 kPa).

Finally, we demonstrated the role of myosin II and focal adhesion kinase (FAK) in SK-HEP-1 cell alignment; chemical inhibition with blebbistatin (MII inhibitor) and PF-573228 (FAK inhibitor) abolished multicellular actin fibre alignment ($n = 3$, $p<0.001$, one-way ANOVA with Tukey's test).

In conclusion, inert viscosity enhancers, in the absence of a flow, produce typical effects observed in shear stress such as enhanced migration, cell alignment, and the activation of mechanotransduction pathways. These results indicate that the use of inert viscous polymers may affect the behaviour of transplanted as well as the host's own cells.

New trimethoxysilylated end-capped hyper branched polyglycidol/polycaprolactam (HBPG/PCL) copolymers for cell delivery and tissue repair

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New trimethoxysilylated end-capped hyper-branched polyglycidol/polycaprolactam PCL-HBPG/SiHBPG copolymers have been successfully synthesised and utilized for development of injectable gels that are able to be covalently crosslinked *in vivo* and give a tissue scaffold capable of supporting load and facilitating regeneration of native tissue. The copolymers were prepared by an anionic polymerization of glycidol and 3-glycidyloxypropyltrimethoxysilane using PCL diol 2000 as a macroinitiator. A number of polymerizations were carried out to prepare PCL-HBPG/SiHBPG triblock copolymers with HBPG and SiHBPG contents varying from 50 to 90 mol% and from 1.0 to 5.0 mol%, respectively. The crystallization, melting and thermo degradation behaviour of these copolymers were investigated in details in order to understand the polymer phase behaviour and the formation of ordered morphologies in solutions and in gels.

The aqueous solution behaviour of the copolymers was studied over a wide temperature and concentration intervals by critical micellization concentration, CMC determination, turbidimetry and small angle X-ray scattering, SAXS. The covalently-linked PCL-HBPG/SiHBPG scaffolds that exhibit gradually increasing mechanical strength was formed *in vivo* from physically crosslinked HBPG/PCL particles in a safe and effective manner and in the absence of UV-radiation. They feature tuneable elastic modulus values and undergo enzyme-triggered disassembly which depends strongly upon scaffold composition and structure. No reduction in viability of chondrocyte cells was seen after 24 and 48 h of incubation. The increase in PCL and SiHBPG content improved the viscoelastic properties of the gels.

Mitochondria targeting Iridium complexes initiate cell death in cancer stem cells

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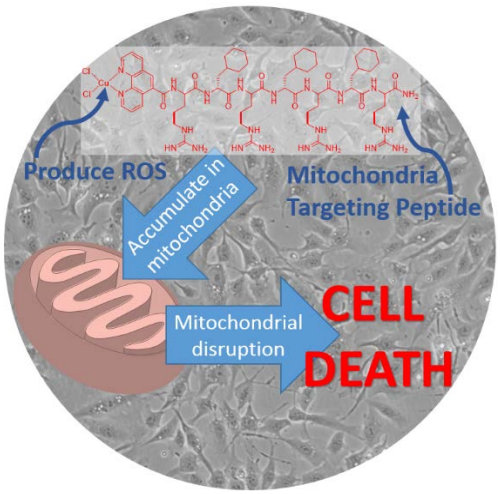
Cancer stem cells (CSCs) are a small population of cells present in many solid tumours which are able to divide asymmetrically resulting in self-renewal in addition to differentiated mature cells of any tissue type.[1] CSCs are resistant to traditional chemotherapy and radiotherapy, therefore they are thought to be partially responsible for cancer relapse. Mitochondrial analysis on various tumour populations have found higher mitochondrial mass in CSCs compared to bulk cancer cells, over-expression of mitochondrial proteins, and key mitochondrial features were distinctly different when compared to their corresponding non-CSCs.[2,3] Therefore, targeting mitochondria and inducing mitochondrial metabolism disruption, altering the reactive oxygen species (ROS) levels, or causing mitochondrial membrane depolarisation offers a viable tactic to activate cell death in CSCs.

Most CSC-specific compounds under current investigation are organic, however recently our group has shown that copper, nickel and cobalt complexes are also capable of removing CSCs in vitro.[4–7] Due to the dense double phospholipid membrane and negative potential across the mitochondrial membrane, positively charged lipophilic compounds preferentially accumulate in mitochondria. Utilising a charged metal centre and a mitochondrial penetrating peptide or lipophilic ligands it is possible to synthesise coordination metal complexes capable of targeting mitochondria. Here we present the cytotoxicity properties of mitochondria penetrating metalloprotein and the mechanism of action.



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Direct cell labelling with ^{89}Zr(oxine)$_4$ allows *in vivo* PET tracking of gamma-delta T-cells

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Introduction: The development and clinical efficacy of cell-based therapies could be greatly improved by *in vivo* cell tracking solutions. Gamma-delta (γδ) T cells are a subset of immune cells with potent anti-tumour activity and have been used successfully in early clinical trials in cancer immunotherapy (1). Here we evaluated the suitability of the recently developed PET tracer ^{89}Zr(oxine)$_4$ ($t_{1/2}$ = 78.4 h) (2) for tracking human γδ-T cells in a xenograft breast cancer model, using PEGylated liposomal alendronate (PLA) to attract γδ-T cells towards tumour tissue (3).

Methods: Human γδ-T cells were isolated from peripheral blood and expanded *in vitro* using zoledronate and IL-2. MBA-MB-231.hNIS-GFP cells were injected subcutaneously in female SCID/beige mice. For tumour sensitisation, one group (*n*=3) was administered PLA (5 mg/kg) 4 days prior to γδ-T cell injection. Another group (*n*=3) was administered placebo liposomes. On day 0, γδ-T cells were radiolabelled with ^{89}Zr(oxine)$_4$ for 20 min and injected in the tail vein ($11x10^6$ cells/animal). Animals were imaged by PET/CT on days 0, 2 and 7. Biodistribution studies were performed on day 7.

Results: γδ-T cells were radiolabelled with 35 % efficiency and showed the expected biodistribution/pharmacokinetics. No adverse effects were observed in the animals over the course of the study. Accumulation of γδ-T cells in tumours was demonstrated by PET/CT and *ex vivo* biodistribution studies. Tumour uptake was higher in the PLA-treated group than in control groups (γδ+PLA: 2.42 ± 0.41 %ID/g versus γδ alone: 1.33 ± 0.31 %ID/g (*n*=3, p=0.013; 1-way ANOVA) and damaged γδ: 0.98 ± 0.18 %ID/g (*n*=3, p=0.003)).

Conclusion: We demonstrated that ^{89}Zr(oxine)$_4$ allows efficient delivery of ^{89}Zr to γδ-T cells and sufficient retention for *in vivo* tracking by PET over 7 days. Accumulation in tumour tissue was enhanced by pretreatment with aminobisphosphonate-loaded liposomes.

References:

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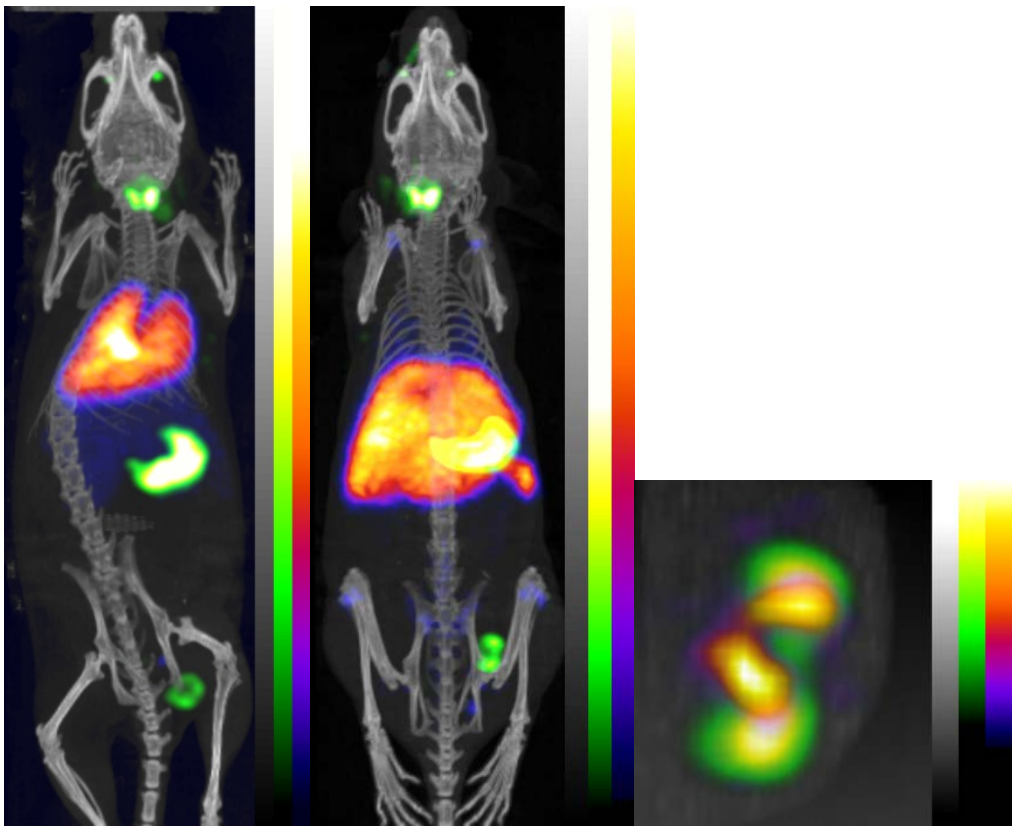

Figure 1: PET tracking of γδ-T cells (^{89}Zr, red scale) and SPECT imaging ($^{99m}TcO_4$, green scale) of MDA-MB-231.hNIS-GFP tumour (T) and endogenous hNIS expressing organs (Thyroid/Salivary glands and Stomach) for up to 7 days. Only 1h and 2d maximum intensity projection (MIP) images post cell injection shown. Lu: lungs, L: liver, Sp: spleen. Enlarged: tumour showing intratumoural biodistribution of ^{89}Zr (γδ-T cells) and ^{99m}Tc (tumour cells) 48 h after γδ-T cell injection