

A Polymer that Binds Bacteria

Professor Ian Douglas, School of Clinical Dentistry, University of Sheffield

Abstract

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Our aim was to synthesize responsive polymers that could bind to bacteria and give an alarm signal above a threshold number of bacteria. Although the signal aspects have not yet been fully achieved, serendipitously we have created a polymer that binds to bacteria and collapses around them, assisting in their removal from infected skin wounds.

The polymer is branched poly(*isopropylacrylamide*), (BPNIPAM), to which the antibiotics vancomycin (BPNIPAM-VAN) and modified polymyxin-B (BPNIPAM-PMX-B) have been coupled. Vancomycin binds Gram-positive bacteria (such as *Staphylococcus aureus*) and polymyxin-B binds Gram-negative (such as *Pseudomonas aeruginosa*). Binding of bacteria at 37°C causes the polymers to become hydrophobic and collapse out of solution, with the bacteria attached.

We have recently developed a 3D model of wounded human skin that we have infected with two species of bacteria. Infection resulted in loss of the epidermal layer and degradation of the extracellular matrix. Application of the polymers in solution caused binding of the bacteria allowing them to be removed by simple washing. We also synthesised the polymers as a coating on a hydrogel support, which when applied to the infected skin and removed also removed bacteria. A time course study showed that the majority of bound bacteria remain viable, thus the polymers do not contribute to antibiotic resistance nor are they simply a topical application of antibiotic.

In conclusion BPNIPAM-VAN and –PMX-B are novel branched polymers sensitive to the presence of Gram-positive and Gram-negative bacteria respectively. These polymers can effectively reduce the bacteria in infected tissue engineered human skin by simple application and removal of the polymers, either in soluble form or coated onto a biocompatible hydrogel membrane. Such polymers could be used to coat wound dressings to aid in the management of chronically infected skin wounds

Biography

Ian Douglas graduated BSc and PhD in Bacteriology from the University of Birmingham. After a postdoctoral position at University of Sheffield working on bacterial cell wall structure, he took up a research fellowship at the Royal College of Surgeons Dental Research Unit to work on development of a caries vaccine. In 1983 he moved back to Sheffield to take up a lectureship in oral microbiology in the Dental School, where he is now Professor. He is also Director of Research for the Dental School and Faculty Lead for Postgraduate Research. For 15 years, he provided a clinical diagnostic oral microbiology service to the Trent Region.

His main areas of research interest are the mechanisms of disease caused by oral bacteria and the development of antimicrobial systems and agents.

New Nanostructured Materials for Devices and Tissue Engineering

Professor Paul Hatton, School of Clinical Dentistry, University of Sheffield

Abstract

Nanostructured composites may exhibit significantly enhanced properties and they therefore have the potential to improve the clinical performance of biomaterials used in dentistry and medicine. The first generation of nanocomposites is already being used in restorative dentistry, although whether or not they offer any real advantages over traditional microfilled and hybrid materials remains subject to debate. There is also evidence that nanostructured materials can overcome some of the challenges presented by the use of composites in orthopaedics. This presentation will first review the claims made regarding structure-property relationships in dental nanocomposites, and then consider how this technology may be developed for the fabrication of biomaterials for orthopaedic applications. The authors are grateful to the EPSRC and Technology Strategy Board for funding, as well as support from industrial collaborators (Smith & Nephew, Ceramisys and eminate Ltd.).

Biography

Paul V Hatton
Professor of Biomaterials Science

Professor Hatton leads the Biomaterials Research Group at the Dental School where he has personal research interests in biomaterials, medical devices and tissue engineering for human skeletal tissue repair. He has published over 70 scientific papers as well as numerous articles and book chapters. He is also active in the promotion of academic-industrial collaboration and technology transfer in the orthopaedic, craniofacial and dental material sectors. See the link below for more details on this and the wider research of the Biomaterials Research Group:

<http://www.biomaterials.group.shef.ac.uk>

Coherent Diffraction Imaging for Life Sciences Microscopy

Dr. Ian L. Pykett, Phasefocus Ltd

Abstract

The Phasefocus Virtual Lens™ is one of a class of Coherent Diffraction Imaging methods based on the Ptychographical Iterative Engine algorithm developed by Professor John Rodenburg of the University of Sheffield. It can generate high definition images of an object without the need for high quality lenses. This is achieved by transferring the task of image formation from physical components to the software algorithm.

The functional capabilities of the Phasefocus Virtual Lens make it particularly suitable for the optical microscopy of specimens that have very low optical contrast (e.g. hydrogels, including contact lenses) or that need to be maintained within a liquid micro-environment (e.g.: live cell preparations.)

The characteristics of the technique that make this possible are:

- Brightfield and high-contrast quantitative phase microscope images from a single acquisition
- Non-contact; very large working distance
- Arbitrarily large fields of view, independent of resolution
- Focus free imaging
 - Post-acquisition interactive selection of any focal plane(s)
 - “3D” through-focal series of entire specimen thickness
- High contrast without staining
- Quantitative refractive index and thickness measurement

Applications in life sciences and optometry will be presented in areas for which current microscopy techniques are not sufficient for user needs.

Biography

Ian Pykett has 25 years' international experience in the commercialisation of high-tech innovations. He was co-founder and CEO of a US start-up that commercialised a medical imaging innovation now in world-wide use. As CTO of a 700-person US high-tech manufacturing firm he grew a three-person R&D group into a spin-out subsidiary valued at £130 million. He is currently CEO of the University of Sheffield spin-out company, Phasefocus Limited, that he co-founded with Professor John Rodenburg in 2006.

A Fellow of both the Institute of Physics and the Institute of Physics and Engineering in Medicine, he has advised on innovation commercialisation and IP asset management for university, RDA, start-up, SME and plc clients, and is Chairman of the UK STFC research council's knowledge transfer panel.

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New Generation Devices for Diagnosing Cervical & other Cancers

Dr Sameer Kothari, Zilico Ltd., Sheffield

Abstract

Zilico Ltd is pioneering new technologies that will allow swifter, more accurate detection of cervical cancer and pre-cancerous conditions.

Cervical cancer affects around 500,000 women worldwide each year and is responsible for 300,000 deaths. Under current screening practices, in the UK a woman will have a Pap smear, or LBC test every three or five years. If the test is positive, she will be referred to a colposcopy clinic for a detailed cervical examination.

Two new products being developed by Zilico will provide “real time” results for women undergoing both these types of test, removing several weeks of waiting for a diagnosis. The tests are safe, painless, and accurate.

The technology used, called electrical impedance spectroscopy, can measure the resistivity of cells and so detect changes as cells progress from normal to precancerous and then to cancerous. This means that assessing smear tests will no longer be a subjective process and the need for diagnostic biopsies will be greatly reduced.

Zilico was formed in 2006 as Aperio Diagnostics Ltd and has focused on developing a commercially manufactured device for the first of its products, aimed at the colposcopy market. Clinical data on 500 women has demonstrated superior performance over existing diagnostic procedures. A multi-centre clinical trial started in April 2009.

Biography

Sameer Kothari – Chief Executive Director, Zilico Limited

Sameer brings over 15 years commercial experience to Zilico. Prior to joining in November 2007 Sameer was CEO of Plasso Technology Ltd, a venture capital-backed company developing research tools and labware for the life sciences. 2007 saw the launch of its first product, EpranEx, a new generation research tool for life scientists. In May 2007, he successfully led a trade sale exit of Plasso to Becton Dickinson Inc.. Prior to joining Plasso, Sameer was a senior executive, with roles in strategy, business development, HR and M&A within Marconi plc. Including a management board position within mainland Europe. Sameer is a fellow of the British American Project and The Winston Churchill Memorial Trust and also mentors two academics looking to commercialise their inventions.

Plastic Viruses

Dr. Beppe Battaglia, University of Sheffield

Abstract

In modern medicine is now widely accepted that how an agent, either a small molecule or large nucleic acid or a protein, is delivered to the appropriate biological site is one of the most important factors in the design of an effective therapy. Efficient administration minimizes side effects, reduces toxicity, and permits better dosage control. As the therapeutic effect of any agent occurs at the molecular level, the effective engineering of a nanovector capable of carrying agents to their therapeutic site will be of enormous value for any therapy. Such a nanovector should have: (i) the ideal size and surface chemistry to cross the various biological barriers, (ii) the ability to encapsulate therapeutic agents, (iii) the ability to circulate in living systems for prolonged periods without being eliminated by the host immune system (stealth) and no toxicity (biocompatibility), (iv) the ability to target specific biological sites, and (v) the ability to release its payload when required.

In the last year we have developed a novel method to encapsulate and delivery a large variety of therapeutic agents based on the natural self-assembly in water of amphiphilic macromolecules. These dual nature compounds comprise both water -soluble and insoluble parts in such a balance that allows the formation of liquid-like (e.g. soft) nanoparticles. These can be designed with geometry, size, surface chemistry, and targeting moieties exquisitely controlled by facile chemical design. Using a reverse engineering approach we have identified a class of such soft nanoparticles that are able to penetrate thick tissues, including solid tumors, and delivery any size molecule within the cell interior. Such approach not only allows the delivery of traditional anticancer drugs with enhanced efficacy, but it allows the delivery of large macromolecules such as antibodies, DNA and RNA, whose use and action has been severely limited by the lack of an effective delivery vector.

Biography

Straight after graduating in Chemical Engineering from the University of Palermo in Italy, Dr Battaglia joined the Particle Process Engineering unit within the Strategic Technology Group in ICI. Here he worked on particle processing as well as the development of novel polymeric drug delivery systems. In 2002, after a year, of Industrial based research he joined Prof Ryan research group at Sheffield University to undertake a PhD in Physical Chemistry. During his doctorate experience Dr Battaglia studied and developed biomimetic nanoparticles based on the self assembly of polymers in water into vesicular structures. In 2006, right after his PhD, he was appointed to a lectureship (assistant professorship) in Bionanotechnology in the department of Engineering Materials at the University of Sheffield. Dr Battaglia is now the Biocompatibles Lecturer in Bionanotechnology and leads a strong group of 20 students and post doctoral fellows. His key research area is in the development and characterization of water-borne nanometer-sized structures and their interactions with live cells/tissues. This involves the synthesis, the physical characterization and the evaluation of such novel systems for applications as: gene and drug delivery systems, tissue engineering and stem cell engineering, and diagnostic devices.

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Quasi-Vivo™ Cell Culture Systems

Providing physiologically Relevant in Vitro Capability to the Academic, Pharmaceutical and Cosmetic industries

J. Malcolm Wilkinson, Kirkstall Ltd., The Sheffield Bioincubator, Sheffield

Abstract

The complexity of the human physiological environment is not replicated in petri dishes or microplates. All cells are sensitive to their micro environment which is rich with cues from neighbouring and distant cells, and from the constant mechanical stimuli due to flow, perfusion and movement. This has been a major limitation to experiments investigating cellular responses in vitro since the complex interplay of mechanical and biochemical factors are absent

Nevertheless, the microwell plate has become the standard in cell culture and is widely used in drug development, toxicology, tissue engineering and stem cell research. Although many researchers and industry accept that classical in vitro experiments offer poor predictive value or mechanistic understanding, there will only be a shift to new technologies for cell culture when these offer significant improvement in function or performance and yet leverage the existing skill set of biologists and technicians. This has been the driving requirement for the development of **Quasi-Vivo™** systems which incorporate modular multicompartmental bioreactor (MCmB) arrays, an easy to use method for cell culture experiments. The new system offers mechanical stimuli in the form of flow and biochemical stimuli from cells placed in connected modules.

This paper will describe the macro, micro and nano scale features of the cell culture chambers that lead to dramatic improvements in cell viability and function compared to older technology. We will also explore how the technology might be used to reduce or replace animal use and reduce bottlenecks in pharmaceutical development.

Biography

J Malcolm Wilkinson

Dr Wilkinson is the Chief Executive Officer of Kirkstall Ltd. Prior to founding Kirkstall Ltd., he managed a high technology consulting company after having had a career in high technology product development both in large corporations as well as start-ups. He has had senior roles in most important functions from R&D through to sales and marketing. In the consulting company he supported spin-outs from Universities and raised over \$15 million from Venture capital and regional development funds. He has a BA from Oxford University, MSc from Southampton and did his PhD research at Middlesex University. He is a member of the IEEE and a Fellow of the Institute of Nanotechnology.

Cell Microarrays for Determining the Mechanism of Action of Candidate Drugs

Dr. Jim Freeth, Retrogenix Limited, Sheffield

Abstract

Drug discovery and development is an inefficient process with extremely high failure rates. It now costs pharmaceutical companies in excess of \$1 billion to bring a new drug onto the market. Two of the main reasons for failure are lack of efficacy and drug toxicity.

At Retrogenix, we are developing a Cell Microarray technology which will be used to help to improve productivity in drug Research and Development. Our technology will allow almost every known human protein to be produced in human cells on an array chip. These arrays can then be used to identify which proteins interact with a given drug, candidate drug, or small molecule.

There are a number of important applications of Cell Microarray technology. Firstly, it will be used to elucidate the 'primary' target of drugs or small molecules for which a desirable disease-relevant biological effect is known, but the mechanism of action is unknown. Secondly, it can be used to identify 'secondary' targets of drugs. This could identify completely new disease opportunities for an existing (and safe) drug. Alternatively, determination of secondary targets can be used to explain, or even predict, drug toxicities such that improved drug can be designed that do not carry this liability.

Biography

Dr Freeth obtained his PhD in 1997 at Manchester University in the field of Molecular Endocrinology. Subsequently he undertook a two-year post-doc in gene therapy jointly between Oxford Biomedica (Oxford UK) and the University of Sheffield. In 2000, Dr Freeth moved to the pharmaceutical company AstraZeneca (AZ), where he developed and delivered several technologies to aid the drug discovery processes for all of AZ's disease areas. In addition, Dr Freeth led projects in specific disease areas, namely in Osteoarthritis and Pain. In September 2008, Dr Freeth left AZ to establish Retrogenix Ltd, in order to address an unmet need in the pharmaceutical industry using Cell Microarray technology. The company is housed with state-of-the-art laboratory facilities at the Sheffield Biocubator.

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Developing 3rd Generation Therapeutic Proteins
Richard Ross, Professor of Endocrinology, University of Sheffield, and Chief Scientific Officer Asterion Ltd.

Abstract

Cytokine hormones have a short plasma half-life and require frequent administration. For example, growth hormone (GH), an anabolic cytokine important for linear growth and normal body composition, involves daily injections. A number of approaches have been taken to create long-acting cytokines, including pegylation and sustained-release formulations but these have the disadvantages of chemical modification, reformulation and, for depot preparations, pain on administration and a dominant early-release profile. There is a need for cytokine formulations that minimise manufacturing costs, have good pharmacokinetic profiles, are easy to administer, and are acceptable to patients. In common with other cytokines, extracellular domain GH receptor circulates as a binding protein and naturally prolongs GH's biological half-life. Thus, like many hormonal systems, binding in the circulation provides an inactive circulating reservoir in equilibrium with active free hormone. We have investigated the biological actions of a ligand-receptor fusion (LR-fusion) of GH with its extracellular domain receptor. Such a genetically engineered LR-fusion protein was purified from mammalian cell culture. In rats the LR-fusion had a 300-times reduced clearance compared to native GH. In hypophysectomised rats daily GH administration promoted a 16% gain in weight over 10 days whereas a single dose of GH promoted no growth. Moreover, an equimolar single administration of the LR-fusion promoted growth for 10 days similar to that seen with daily GH and generated a far greater rise in IGF-I which is responsible for many of the anabolic actions of GH. In solution the LR-fusion exists as both a dimer and monomer. We propose that the LR-fusion forms a reciprocal, head-to-tail dimer that provides a reservoir of inactive hormone as occurs naturally with GH and its binding protein. In conclusion, an LR-fusion of cytokine to its extracellular receptor generates a potent long-acting agonist with exceptional pharmacokinetic properties. This approach could readily be applied to other cytokines.

Biography

Professor Richard JM Ross MBBS MD FRCP

Professor Ross trained in medicine at The Royal London Hospital (1974-1979) and in Endocrinology at St Bartholomew's Hospital, London (1983-1988). He was appointed to Sheffield University in 1995 and is Head of the Unit of Diabetes and Endocrinology. He previously served on the Medical School Council as Head of Section for Endocrinology and Reproduction (2005-2008).

Professor Ross's research and clinical interests are in Pituitary Disease, Transition Endocrinology and the late effects of cancer therapy. His research has yielded some 130 papers in peer reviewed journals, over 50 chapters and two books. He has a particular interest in commercial research. He was a Knowledge Transfer Champion in the university, is a founding Director of Sheffield Health Innovation Centre and is a founding Director of two university spin-out companies; Asterion Ltd and Diurnal Ltd. He has 9 granted patents and personally obtained Orphan Drug Designation from the EMEA for Chronocort; a new therapy for Adrenal Insufficiency.

Viral Vectors: Gene Therapy and Research Application in Neurodegenerative Diseases

Professor Mimoun Azzouz, Department of Neuroscience, University of Sheffield

Abstract

Lentiviral vectors based on the equine infectious anaemia virus (EIAV) have been well characterised and mediate efficient and sustained transgene expression in cells of the nervous system. This presentation will describe the design of EIAV vectors and their application for gene transfer in the nervous system for gene therapy applications in animal models of human neurodegenerative disease such as Amyotrophic Lateral Sclerosis (ALS) and spinal muscular atrophy (SMA).

We have investigated multiple gene transfer strategies aimed at improving motor neuron survival in animal models of motor neuron diseases. We have utilised a rabies-G pseudotyped lentiviral vector expressing the VEGF gene to achieve expression of this neuroprotective factor in spinal motor neurons of SOD1^{G93A} mice – a widely used animal model of ALS. Retrograde VEGF gene delivery delayed the onset and slowed progression of ALS, improved muscle performance, prevented motor neuron death and prolonged survival of SOD1^{G93A} mice. Most notably, VEGF gene transfer increased the life expectancy of ALS mice by 30%, without causing toxic side-effects, thereby achieving one of the highest therapeutic effects reported in the field to date (Azzouz et al., 2004a). We have also investigated the potential for using lentiviral vectors as a method of delivery for RNAi targeted against the human form of SOD-1. Retrograde delivery of Vectors carrying optimised RNAi sequences to motor neurons of the spinal cord and brain stem by non-invasive intramuscular injection resulted in a significant reduction in mutant SOD1 expression *in vivo*. Long term studies investigating the motor performance of SOD1^{G93A} mice following mutant SOD1 silencing revealed a strong therapeutic effect (Ralph et al., 2005).

SMA represents a recessive autosomal disorder and is one of the most common genetic causes of death in childhood. It is caused by mutations or deletion of the telomeric copy (*SMN1*) of the survival motor neuron (*SMN*) gene, leading to depletion in SMN protein levels. The treatment rationale for SMA is to halt or delay the degeneration of MN, but to date there are no effective drug treatments for this disease. Here, we report that Lentiviral vector expressing human *SMN* was successfully used to restore SMN protein level in SMA type 1 fibroblasts. Multiple single injections of a lentiviral vector, expressing *SMN*, in various muscles of SMA mice, restored SMN to MNs, reduced MN death and increased the life expectancy by an average of 3 and 5 days (20% and 38%) compared to LacZ and untreated animals, respectively (Azzouz et al., 2004b). Further extension of survival by SMN expression constructs will likely require a better knowledge of when and/or where high levels of SMN are needed. Additional studies are ongoing to investigate SMN gene replacement in a mild model of SMA (Monani et al. 2003).

The above results are indicative that gene therapy using Lentiviral vectors might prove beneficial in the treatment of motor neuron diseases.

References: Ralph et al., (2005) *Nature Medicine* 11:429-33; Monani et al., (2003) *J. Cell Biol* 160: 41-52; Azzouz et al. (2004a) *Nature* 429: 413-417; Azzouz, M. et al. (2004b); *J.Clin. Invest* 114 : 1726-1731.

Biography

Mimoun Azzouz (MA) is a Professor of Translational Neuroscience at the University of Sheffield and formerly Director of Neurobiology at Oxford BioMedica Ltd. Mimoun Azzouz received his Ph.D. in Pharmacology and Neuropharmacology in 1997 from the University Luis Pasteur of Strasbourg, France. He did his post doc at the Gene Therapy Center in Lausanne, Switzerland from 1997 to 2000. He then worked as Director of Neurobiology at Oxford BioMedica Ltd, Oxford, UK until December 2005. He was also a visiting scientist at Oxford University between 2000 and 2005. MA has led the field internationally in developing and evaluating gene therapy approaches using viral vectors, with the potential for exciting new therapeutic approaches in neurodegenerative diseases. Gene therapy in models of Parkinson's disease (PD) is already translating into human clinical trials and three patients were treated in March 2008.

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Hazard Identification and Risk Management for Novel Medical Technologies

Richard Moore, Institute of Nanotechnology

Abstract

The application of nanotechnology in medicine brings new challenges in relation to the identification of hazards, the estimation of risks arising from those hazards, controlling risks and communicating about them in the right way for different audiences.

The challenge is furthermore compounded by the fact that some products may fall under more than one regulatory system, each of which has different traditions in addressing risk issues.

In this presentation, Richard Moore, who has long experience of risk management as applied to medical devices as a member of the CEN and ISO committees that drew up standards on this subject, will examine some of the key concepts and aspects of hazards and risks that are pertinent to the medicine at the nanoscale as well as highlighting how important this will be for both product safety and communication to both professionals and the public.

Biography

Richard Moore has been responsible for work programmes in the areas of nanomedicine and the lifesciences at the Institute of Nanotechnology (IoN) since the beginning of 2007. This includes running the NanoMedNet nanomedicine network for clinicians and other professionals (www.nanomednet.org) and the development of a modular series of professional training courses and workshops in the field of nanomedicine. He also participates on behalf of IoN in EU research projects under FP6 and FP7 and in national projects concerning nanomedicine, as well as in standardization activities at UK, European and international levels in the field of nanotechnology. He is a frequent presenter on nanomedicine at UK and European conferences and contributes regular articles on nanomedicine to several publications.

Prior to joining the IoN early in 2007, he worked for ten years as Director, Science and Innovation at Eucomed (European Medical Technology Association) in Brussels. At Eucomed, he was responsible for EU-level and international work in the area of standards, environmental legislation, new medical technologies (e.g. human tissue engineering), risk and risk governance, and also for an industry programme of medical technology innovation.

Prior to Eucomed he worked for six years at the European Committee for Standardization, CEN, where he was responsible for the coordination, development and publication of the programme of harmonized European standards supporting European Directives in the field of healthcare.

Richard Moore is a biologist by training and is a Chartered Biologist (CBiol), a Member of the Institute of Biology (MIBiol), a European Professional Biologist (EurProBiol), a Fellow of the Institute of Nanotechnology (FIoN) and a Fellow of the Linnean Society of London (FLS).