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A new microfluidic tool for rapid analysis of protein stability and integrity in bioprocesses

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£424203

Analysis of protein stability is currently too slow and requires too much of an exceedingly valuable biopharmaceutical to be useful in guiding bioprocess development or control. Introducing the first microfluidic method for protein stability testing will reduce sample use and cost of analysis by up to 108-fold over microwell-based analysis. Combined expertise from biochemical engineering and the London Centre for Nanotechnology will enable this analysis with parallelism for up to 1000 samples per day. The new generation of protein-based medicines has rapidly become a \$30billion-a-year industry addressing previously untreatable diseases. They have the potential for much further growth but a principal constraint is the high cost of the manufacturing methods required to preserve the structural integrity of proteins with limited stability. The ability to perform rapid and parallel protein stability characterisation experiments, at the microfluidic scale, is essential to enable: a) the rapid optimisation of therapeutic protein formulations; and b) the real-time monitoring of protein product quality in process-, microwell- and microfluidic scale bioprocess development experiments. Our preliminary research has demonstrated protein stability determination using fluorescence measurements at the microwell scale (Aucamp et al., 2005). The aims of this proposal are to a) explore the fundamentals that impact on measurement accuracy and sensitivity at the microfluidic scale, so as to significantly decrease the sample volumes required for protein stability measurement; b) establish a microfluidic denaturation technique; c) overcome the challenges that will enable broad application to bioprocessing and formulation of biopharmaceutical protein products.

Application of metabolomics profiling of recombinant mammalian cells to bioprocess design

Professor Alan Dickson

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£768109

The diagnosis and treatment of many clinical conditions is dependent on developments of new therapeutic drugs / complex protein molecules that require production by mammalian cells in culture. Advances in genetic engineering and understanding in gene expression has made it possible to turn mammalian cells in defined culture conditions into "factories" to permit harvest of the valuable therapeutic protein drugs. Hence it is possible to introduce into mammalian cells in culture a gene that will direct synthesise the desired drug and allow the mammalian cells to perform all the necessary reactions required to generate the therapeutic protein. Some of the drugs generated by these approaches are household names (eg insulin and clot-buster drugs) and others are not (eg antibodies), however all are critical and essential components in treatments for many life-threatening clinical conditions. A key problem is that the development and generation of sufficient amounts of therapeutic protein drugs is limited by the capacity to generate sufficient production from the cell cultures. Consequently, the investment of development and process time to harvest sufficient amounts of therapeutic proteins makes the process costly and these are expensive drugs. Despite significant effort by industrial and academic researchers we still do not fully understand the factors that limit production by the cell culture system and such information will be essential if we are to devise means to increase production. The unit cost for certain of these therapeutic protein drugs has implications for availability for treatment and for certain "difficult-to-make" drugs financial implications may prevent the drug being developed for the market. This proposal addresses this problem and seeks to use novel approaches to ask how we can improve the process of therapeutic protein formation by mammalian cells in culture. Our approach is to take a wide vision of the events within cells that limit formation of the desired drug. Too frequently experimental approaches to study the functions of cells focus on one specific aspect or examine functions in the cell for which there is no direct certainty of involvement in the processes under study. We are building complex models that will determine how the many factors in the cell interact to define how production of the therapeutic protein is controlled. We will then test the model in response to conditions that we impose in cells and, from this, determine if there is a means to identify those cells in a population with desirable characteristics that will give excellent production or if we can engineer these functions into cells. The research team in this programme have come together from distinctive areas of research, allowing a fusion of technologies to emerge from the interactions. The fusion of analytical scientists, cell biologists, chemical engineers and mathematical modellers generates a novel team-based approach to an industrially- (and, ultimately) clinically-relevant issue. The team will be part of a wider network of scientists (in academic and industrial groups) who are part of the Bioprocessing Research for Industry Club, the government/industrial initiative to maintain the UK research strength in this area.

BRIC: Packaging cell lines for inherently manufacturable viral vectors

Professor Nigel Slater	University of Cambridge	£230652
Professor Farzin Farzaneh	Kings College London	£251318
<p>Viruses dominate overwhelmingly the types of vectors currently being tested in clinical gene therapy trials and of these retro- and lentiviruses are the most numerous. Until recently two technological problems have hampered progress in gene therapy; production of high titre clinical grade virus and efficient tissue specific targeting. Research at Cambridge and King's College London has addressed the former and led to the development of a novel lentiviral vector packaging cell line in which manufacturability is built into the genome of the packaging cell and co-expressed on the surface of the viruses produced thereafter. We initially used simple retroviral vectors, and latterly the more complex lentiviral vectors based on a core of HIV-1, and have developed strategies for increasing the titre by several orders of magnitude. This is an active area of research amongst which our preliminary work with novel chromatographic techniques and paramagnetic particles set the foundation for a practical and efficient alternative technique to cumbersome ultracentrifugal concentration. For lentiviral vectors we engineered a new producer cell type that provides a biotin tag amenable to various lentiviral vectors produced from these cells using either VSV-G or MLV amphotropic envelopes. We have shown that these bio-lentiviral vectors are produced in the normal manner and only require the presence of biotin in the culture medium to manifest their affinity for streptavidin. Vectors can thus be retained on streptavidin Paramagnetic Magnospheres for infection, or eluted from streptavidin adsorbents. This cell line allows the capture of multiple envelope pseudotypes of lentiviral or MLV derived vectors, enabling production and concentration to titres that are several orders of magnitude higher. Using this scalable protocol we have concentrated lentivirus in excess of 4500-fold in only 3 h and have provided titers for both VSV-G and MLV amphotropic envelope pseudotypes of 1010 IU/ml. However, these viruses could not be easily eluted from adsorbents and required the addition of biotin to the growth medium of the packaging cells. This proposal aims to express the alternative desthiobiotin ligand on the surface of lentiviruses in such a way that elution from adsorbents may be more readily preformed to give higher process yields and the addition of an affinity ligand binding precursor to growth medium is avoided.</p>		

Characterization of post-transcriptional constraints that determine rP yield during bioprocessing in mammalian cells

Mark Smales	University of Kent	£987761
<p>We all have an in-built defence mechanism to respond to infection when our body recognises a foreign 'invader'. A type of cell known as a B cell responds to infection by changing into an antibody-producing cell. Antibodies are proteins that work by attacking the foreign invader and destroying it, clearing infection by removing the foreign agent. Because antibodies are our body's natural defence against disease, many new antibody type drugs are being developed to help treat a number of human diseases such as cancer. These antibodies are usually produced by cells kept in a culturing solution under defined conditions. The problem is that these antibodies must be in a special shape; otherwise they do not work. The cells used to produce such antibodies have a very complex set of machinery to make the antibodies and put their components together into the right shape. This works very well when the cell is not expected to make much of the antibody in question. However, the cells we use to make antibodies are much less efficient at producing these drugs when we try and produce more of the product. As a result, we are not able to produce enough of these drugs and the cost and demand for them is therefore high e.g. the breast cancer drug Herceptin. The root of the problem is that when cells are asked to produce much more protein to meet our needs the machinery can no longer cope / the cells sometimes die or else don't produce antibodies of the right shape, of no clinical use whatsoever. It is largely agreed that this problem will become even serious as further antibody-based drugs are developed. The research proposed here will investigate how the cell machinery for making proteins works and examine whether, and in what ways, it can be manipulated to produce more antibody. We want to determine the different parts of this machinery that are limiting in terms of making the antibody, and then investigate how these parts work together to ultimately produce the antibody of interest. At present it is unknown if this is possible, and the process is poorly understood in the mammalian cells presently used to produce antibodies. We will employ a combination of new state-of-the-art technologies and approaches to take apart the antibody assembly line in mammalian cells in a step-wise manner, and then using the information gathered will determine the relationship between each step of the assembly process. Ultimately this should enable the manipulation of cells to change the balance of each step in the assembly line to produce more of the target antibody drug at reduced cost and higher quality. As stated above, this is extremely important as it is expected that with an increasing number of protein 'drugs' being developed we will lack the capability of producing large enough amounts to meet the required demand for these new drugs for the majority, as opposed to for those who can afford what must currently remain prohibitively expensive, but very effective, medicines.</p>		

A Novel Bioreactor System for Manufacturing in Stem Cell Therapy and Tissue Engineering

Professor Julian Chaudhuri	University of Bath	£186350
Professor Richard Oreffo	University of Southampton	£185475
<p>The emergence of regenerative medicine offers the potential for new therapies and procedures for diseases and injuries that cannot currently be effectively treated. Regenerative medicine involves the use of living cells and other biological molecules to restore damaged structure and function in human organs and tissues. In addition, the recent excitement arising from the discovery and potential uses of stem cells makes it timely to investigate how stem cell research can be used to treat patients. One of the key issues is how to produce enough living cells (including the very rare stem cells) that have the correct function for these new therapies. The current laboratory cell culture procedures are not efficient, nor are they standardised and cannot meet the current clinical needs. This project aims to address this acute issue by the development of a new method of efficiently culturing stem cells and other human cells to give enough cells required to treat patients.</p> <p>This system will be based on the idea of growing cells using a biomaterial derived from seaweed (alginate) that allows the cells to grow and develop normally. In this work, we will decide what effects the key features of the culture system will have on the growth and function of both human stem cells, and also specific human cell types (eg bone cells). As part of our experiments we will measure how the cells grow and behave in the different culture environments, and compare this to conventional methods.</p>		

Identification of novel signal transducers in the mammalian unfolded protein response		
Dr Martin Schröder	Durham University	£677697
<p>Human proteins produced in other organisms, i.e. bacteria or mammalian cell cultures are called recombinant or heterologous proteins. These proteins have many applications in industry and medicine. They are safer to administer in the clinic than their native counterparts purified from i.e. animals. Single point mutations in a protein, i.e. isolated from pigs, that deviate from the human sequence, can have serious side effects when the protein is administered to humans. Further, their posttranslational modifications, i.e. their glycosylation pattern, are similar to those found in humans. Again, minor deviations in the glycosylation pattern between an animal and a human protein can seriously affected the performance of the protein in the human body. Finally, the risk of contamination of the protein with infectious agents is much easier controlled in production processes for recombinant proteins compared to isolation of the protein from animals. Production processes for recombinant proteins for use in the clinic rely on mammalian cell lines. Growth conditions and sterility requirements for these production processes make them expensive. Further, the number of recombinant proteins for which there is a demand on a multikilogram per year scale is rapidly growing, requiring the development of large scale (several cubic metres) production processes. The cost efficiency of a production process often determines if a recombinant protein makes it to the market and to the clinic. Several strategies have been devised to improve product yield and in turn the cost effectiveness of these production processes. To date, the rate-limiting step for production of recombinant proteins is folding into their native, active, and non-immunogenic conformation. Protein folding is assisted by helper proteins that shield a folding protein from its environment, called chaperones, and that catalyse a subset of protein folding reactions, called foldases. Recent basic research has identified signal transduction pathways that sense the folding status of a protein, and that activate expression of these helper proteins to increase the efficiency of protein folding in response to this stress situation. Genetic engineering of these signalling pathways promises to alleviate the protein folding bottleneck in recombinant protein production. However, our current understanding of these signal transduction pathways is still limited. Based on our current knowledge the outcome of engineering of these pathways on recombinant protein production is unpredictable. To address this problem we propose to characterize these pathways on a genomic scale to define their complete substrate spectra by using recently developed genomic technologies to monitor the complete mRNA and protein population of a cell. In addition, we will exploit unique biochemical characteristics of these signalling pathways to focus our study on the pathways in question. In this way we will target three unique signal transduction mechanisms, non-spliceosomal splicing of mRNAs, preferentially translated mRNAs when translation of the majority of mRNAs is inhibited, and proteolytic activation of ER membrane proteins. We anticipate to identify new proteins involved in this response to protein unfolding. We will characterise the role of new genes involved in these signalling pathways identified in our study by characterising their role in chaperone and foldase expression, and survival of ER stress. Furthermore, through defining the complete substrate spectra of these pathways we will enable the engineering of these pathways with predictable outcomes on cell specific protein production and cell viability.</p>		

Microbial physiology underpinning the production of difficult recombinant proteins		
Professor Jeffrey Cole	University of Birmingham	£386309

Most people appreciate the need for pharmaceutical companies to develop new vaccines to prevent disease, or drugs to combat illness, not only for humans, but also for our domestic animals. In this context, biotechnology and genetic engineering are clearly tools to be used for the benefit of society. The development of new vaccines and drugs often depends upon the ability of bioprocessing companies to harness a cell factory to produce one or more target proteins. In many cases this will mean using simple but safe bacteria to generate the required product. Bacteria can be taught to synthesise almost any protein, once they have inherited the correct DNA coding sequence. However, under the conditions they are grown in the laboratory, they are often unable to assemble the protein correctly, so the product is useless. Sometimes it is better to make less product more slowly so that it is not toxic to the cell. In other cases, extra copies of helpful genes need to be transferred to the cell factory so it can assemble and modify the target protein after it has been generated from the genetic information provided. In this project, we will discover why some proteins are so difficult to make, and how to help bacteria make them more efficiently. We will start by making a protein that is located on the outer surface of the bacterium that causes the sexually transmitted disease, gonorrhoea. It is also found in bacteria that cause teenage meningitis (the so-called "kissing disease"). Vaccines are required for both gonorrhoea and type B meningitis. We will discover how to prevent the accumulation of useless product, and how to make authentic protein. The UK bioprocessing companies who have formed a club to support this type of research will then be invited to challenge us with one of their unsolved problems. This will allow us to test whether there are general rules that must be followed if other difficult proteins are to be generated for the benefit of human health.

Modelling cellular processes underpinning recombinant monoclonal antibody production by mammalian cells

Professor David James

University of Sheffield

£524266

This proposal is concerned with "bioprocessing". Bioprocessing collectively describes the range of manufacturing processes that enable the production of new biological medicines. You may be familiar with the one of the first biological medicines produced by recombinant DNA technology - a small protein called insulin. Insulin is now used very successfully to treat an increasingly common metabolic disease, diabetes. Before insulin, diabetics suffered a short life fraught with serious medical complications. This project is targeted at the production of other high-value therapeutic proteins by genetically engineered mammalian cells in culture, specifically monoclonal antibodies. In the body, natural antibodies present in the blood play an important role in our immune system: They target disease-causing microbes and foreign substances for removal. Recombinant monoclonal antibodies, being almost identical to natural antibodies, are specifically designed to target diseased cells. Unlike traditional small-molecule medicines such as penicillin and paracetamol, monoclonal antibody biopharmaceuticals are large, complex and relatively fragile proteins which have to be produced by living mammalian cells in culture, genetically engineered to produce the recombinant protein product. They are proving to be highly successful treatments for serious diseases such as rheumatoid arthritis and a range of cancers. It is anticipated that within the next five to ten years up to fifty percent of all drugs in development will be biopharmaceuticals; a very substantial proportion recombinant proteins produced by mammalian cells in culture. Since the first recombinant protein medicines produced by genetically engineered mammalian cells in culture were licensed as therapeutics over 25 years ago, we have learnt to substantially increase the productivity of biopharmaceutical manufacturing processes (bioprocesses). However, they are still complicated and expensive, and industry has to undertake time-consuming screening processes to find engineered cells making adequate amounts of recombinant protein. To date, the output of industrial bioprocesses has predominantly been increased by gradually improving the growth of producer cells in culture, and not by engineering each cell to make the product more efficiently. This is important, because if we knew how to instruct or programme the cell factory appropriately, we could substantially improve the productivity of manufacturing processes and decrease the time it takes to generate a productive cell culture. However this is not a simple problem. The cell utilises and coordinates a diverse range of its complex machinery to turn, for example, recombinant monoclonal antibody genes in its nucleus into a fully folded protein which can be secreted out of the cell. How can we understand this cellular "production line" well enough so that we can rationally implement strategies to improve flux from recombinant genes to protein product? In this project we will implement a novel, multidisciplinary combination of technical approaches to answer this question; mathematical modelling, gene expression, molecular cell biology, protein analysis and cell culture. We believe this is crucial - an integrated mathematical bioscience approach can massively increase the information content and utility of biological measurements and enable us to understand cellular processes from a systems control perspective. This project will, for the first time, provide a quantitative understanding of the cell factory on which to rationally build strategies to increase the productivity of therapeutic monoclonal antibody production systems. Without this knowledge, cell culture engineering will largely remain based on trial and error.

Strategy for the consistent preparation of sufficient non-viral large chromosomal vectors for biopharmaceutical applications

Dr Eli Keshavarz-Moore	University College London	£377945
<p>Modern medicines and therapies are becoming increasingly complex and specific for a particular disease or group of patients. One very specific type of therapy is Gene Therapy. Gene therapy is the use of genes as medicines. These genes can be delivered to patients either by the use of genetically modified viruses to carry the genes or by using non-viral methods which employ circular DNA molecules called plasmids isolated from bacteria. Both techniques are still in their infancy but are already promising huge medical advances in vaccination, cancer therapy and the correction of genetic disorders. However, viruses suffer from several drawbacks including safety considerations and limited to carry large genetic information. On the other hand large plasmid DNA molecules called BACs (Bacterial Artificial Chromosomes) can be modified to incorporate a wide range of important control regions which allow expression of the gene in the correct tissue and at the correct time. This larger size poses a scientific and industrial challenge because the size limits the amount that can be made in a single bacterium, in addition, the size also calls for special processing considerations because BACs are fragile. The challenge is to be able to prepare the BAC molecules in the amounts needed to treat the numbers of people who could benefit from these medicines. We need to investigate then develop methods of making the DNA molecules at large scales in the biomanufacturing industry. The quality of the DNA molecules is also paramount. They need to be in the form that is most appropriate for delivering to humans (and animals) and free of contaminating material. The research we propose will enable industry to make DNA molecules that can be made at the large scales. The proposed research outcomes will allow others involved in gene therapy to prepare large DNA molecules for the treatment of cancers, for vaccines and to correct genetic disorders The science proposed will also allow other researchers in related disciplines to benefit from being able to make and manipulate large DNA constructs .We will also explore ways of making the DNA in the correct three dimensional form needed for efficient uptake into cells so that the DNA is an effective medicine.</p>		

Total £5,068,335