News from the UK Branch of the ETCS

ETCS Meeting, York, March 21st

The UK branch of the European Tissue Culture Society (ETCS-UK) facilitated a highly successful 2-day symposium at the Central Science Laboratories, York, March 21-22. The symposium topic was "Models of Stem Cell Biology", organised by Dr. Paul Genever and Dr. Claire Varley, University of York, together with Dr. Andy Scutt from the University of Sheffield, who was responsible for the programme. The ECACC provided administrative support and sponsored the abstracts/programme book. Sigma-Aldrich and ECACC exhibited at the meeting.

The programme was divided into four Sessions, each of four presentations, that focused on:

1. Stem Cells: their biology, differentiation and culture requirements.
2. 3-Dimensional Cell Culture: neuronal stem cells, retinal cells and bladder epithelial cells.
3. Tissue-specific Differentiation: chondroprogenitor cells, muscle cells and bladder epithelial cells.
4. Transdifferentiation: stem cells/progenitor cells in the context of liver function, chondrocytes and peritoneal mesothelial cells.

The Sessions were enabled by an impressive group of invited speakers with established reputations in their specialist fields. All were excellent and the standard of science was extremely high. ETCS-UK extends particular thanks to Dr. Steven Minger, who "jetted" in from a previous scientific meeting in the nick of time to deliver his presentation, then immediately "jetted" out to another meeting. Details of the contributors can be found on the ETCS-UK pages of the ECACC website.

Professor Ian Mackenzie (University of London) chaired a thought-provoking workshop on "Cancer Stem Cells", primed by presentations from himself together with Dr. Anne Collins (University of York). The central theme was that only a small proportion of tumour cells are tumourigenic, and that this sub-population has stem cell-like characteristics. These "cancer stem cells" are capable of generating new clones containing additional stem cells as well as regenerating phenotypically mixed populations of non-clonogenic cells present in the original tumour.

The symposium was well attended by approximately 100 delegates, and the organisers were congratulated. ETCS-UK has maintained a long tradition of good quality scientific meetings and the next meeting is being planned. Monitor the ECACC website for further news.

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Getting Tissue Culture Closer to Life

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For the most part, primary tissue culture of any given cell type entails dissociation of the tissue in which that cell naturally resides and plating it out onto a suitable substratum using a medium that is compatible with its growth. Such schedules commonly include a procedure for enriching or ideally for purifying the cell of interest from the mixture of cells released from the original tissue. Skeletal muscle has been an interesting variant on this general approach, since the mature muscle cells are very delicate once removed from their surroundings and do not survive most isolation procedures. Muscle tissue is normally cultured from the precursor cells present in muscle, which are generally thought to be synonymous with the satellite cells that lie sandwiched between the plasmalemma of the muscle fibre and the overlying basement membrane. The subsequent proliferation, differentiation and fusion of the muscle precursors to form multinucleate 'myotubes' has become one of the more popular models of tissue differentiation, being quite reproducible in some hands.

As a model of muscle in the body however, this type of culture has a number of shortcomings. Some of these arise from the failure of the myotubes to progress to the later stages of differentiation probably reflecting our inability to properly reproduce the normal *in vivo* environment in which the muscle fibres are innervated, vascularized and linked via tendons to a compliant skeletal system on which, their normal function would require them to exert tension. Other deficiencies are to do with the uncertainty of provenance of cells isolated from muscle by standard enzymatic disaggregation techniques.

This uncertainty raises 3 types of question. Are all of the myogenic cells derived from satellite cells? Are all satellite cells myogenic? How representative are the cells in culture of the myogenic population responsible for myogenesis *in vivo*? These can all be answered, in large part, by using muscle fibres isolated cleanly from the muscle by digestion with carefully selected batches of collagenase as starting material for culture [1]. If such preparations are made cleanly, using only fibres that are not hyper-contracted as starting material, it is possible to eliminate contamination by non-muscle cells and to be sure that all cultures start only from satellite cells. The fibre, being the standard unit of muscle, forms a basis of comparison for calculating differences in yield between muscles, or animals, or treatments, or other variables. It is also possible to characterize the starting muscle fibres in terms of the numbers of satellite cells they carry, for comparison with how many myogenic cells they produce within a chosen time period [2]. By such means, tissue culture can be used as a monitor of the myogenic qualities of the muscle from which they were derived and to make quantitative inferences that relate directly to myogenesis *in vivo*. A further aspect of this type of preparation, is that the freshly prepared fibre provides a highly synchronised model of activation of a quiescent precursor into a pathway involving both self replacement and production of differentiated tissue [3].

References

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A Novel Process for Cell Microencapsulation

'Tandem' Hydrogels

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Introduction

The ability to introduce cultured cells into a biological system without concerns for adverse biological reactions or mechanical damage offers a number of potential medical, technical and economic benefits. The protection of "guest" cells from a host immune system (immunoisolation), or from mechanical damage in a bioreactor, can be approached by encapsulating the cellular material within a membrane or a capsular matrix, that allows nutrients, wastes, and therapeutic products to permeate and diffuse freely, while acting as a barrier to external influences such as mediators of a host immune system. We are specifically interested in microencapsulation, where sub-mm spherical, hydrogel-based constructs (capsules if hollow, beads if solid) are used for entrapping individual or aggregated cells. Such systems are commonly used for cell transplantation purposes[1].

Materials for Cell Encapsulation

Calcium alginate has been the most commonly used material for many years. Calcium alginate is a polysaccharide naturally occurring in its sodium salt form. It produces gels through a particularly cell-friendly process, which is based on a calcium-induced ionotropic gelation, a physical process where multivalent cations (such as calcium) bridge and thus physically cross-link polymer chains[2, 3]. This process has the advantage of rapid kinetics: cells can be dispersed in an alginate solution, which immediately solidifies when exposed to a calcium solution. However, alginate suffers some drawbacks. Variability in its chemical structure affects the strength and homogeneity of the gel structure, the reversibility of the gel (kept together by extractable calcium ions), and the frequent presence of endotoxins. Improved purification procedures and polyelectrolyte complexation on the surface of the alginate beads have partially overcome these problems. However, appropriately designed synthetic materials may provide better alternatives.

Chemically cross-linked synthetic hydrogels comprise a type of material that is inherently irreversible and possibly tougher than alginates. Their method of manufacture is also more controllable. A major disadvantage, however, hampers the use of such systems: even if cell-benign reactions are used for cross-linking, their kinetics is generally not compatible with processing techniques that require an 'instantaneous' gelation. Chemical reactions are "slow" compared to purely physical interactions.

Tandem Hydrogels

In the preparation of calcium alginate (and most other physical gels) there is a substantial overlap between morphogenesis of the material (the 'gelation' or sol-gel process) and development of its final mechanical and transport properties. We have realised that these two aspects can be easily decoupled: for example, by combining a quick physical gelation with a slower chemical cross-linking, it is possible to obtain a solid material with the rapid kinetics typical of physical processes. On the other hand, after an appropriate incubation time, superior mechanical properties and irreversibility are achieved by the formation of a network of covalent bonds. We have described the use of these two different hardening processes as tandem gelation because, while each independently provides a result similar to a hydrogel, together they interact synergistically to allow improved performance.

Specifically, we have used synthetic poly(ethylene glycol)-poly(propylene glycol) triblock copolymers, known as Pluronics or Poloxamers when linear, as Tetronics or Poloxamines when 4-armed, which present a reverse thermal gelation in water solution[4, 5]: The result is soluble in cold water, and forms gels at a range of physiological temperatures. By introducing an appropriate number of reactive functional groups at the chain termini (thiols and electron-poor carbon-carbon double bonds), such polymers can also undergo an irreversible gelation through the occurrence of a Michael-type addition cross-linking reaction[6, 7] (Figure 1).

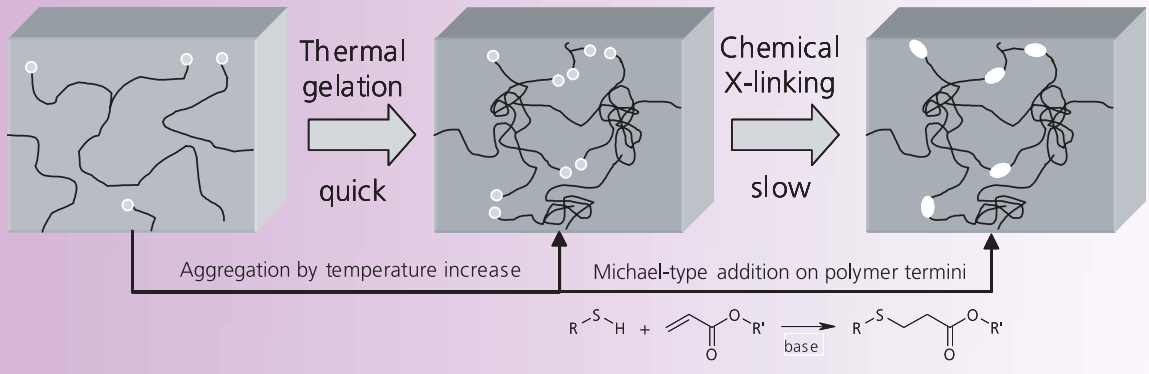

Figure 1. Schematic view of a 'tandem' process. The physical gelation determines a quick hardening process; the material is then cured with a slower kinetics.

Initially, the polymers can be dissolved in water at low temperature (5-10°C) and controlled pH (6.8-7.2). Under such conditions the physical gelation is avoided and the chemical reactions take place at a negligible rate, because Michael-type reaction kinetics is pH-dependent. Upon exposure to a physiological environment (37°C and pH7.4), these solutions rapidly gel and with a slower kinetics chemically cross-link[6, 7].

This 'tandem' gelation mechanism allows to obtain materials resembling alginate in a) hardening kinetics, due to the fast thermal gelation, b) mechanical and transport properties, due to the possibility of adjusting the gel mesh size in the chemical curing, c) biocompatibility, due to the PEG content and the mild conditions of the Michael-type addition, that proceeds smoothly and quantitatively in physiological conditions without generation of toxic by-products or other undesired effects on cell metabolism[7].

The processing conditions have been optimised for the production of microbeads and microcapsules (Figure 2), generated in a commercial jet-breakup encapsulator in order to achieve a tight size distribution. Preliminary experiments for the entrapment of CHO cells have showed that they entirely preserve their viability after encapsulation[8].

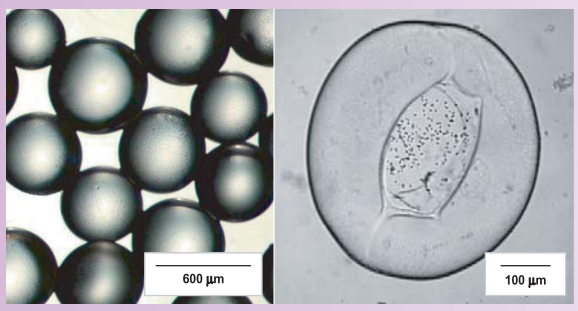

Figure 2. *A.*Collection of regularly shaped Tetronic gel micro-beads (diameter 500÷600µm) photographed after removal of the suspending liquid bath. *B.* Tetronic gel capsule (average shell diameter 900µm) loaded with viable CHO cells. The capsules are produced employing the double nozzle technique so that the gel material is present only in the shell and cells are confined in the liquid core of the particle.

References

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Join the European Tissue Culture Society

Tissue culture is a research tool that has application across a broad spectrum of scientific disciplines. Rarely, now is it an end in itself and essential cell culture skills are often subverted to the primary research aims. There are numerous examples of research programmes that are impeded or invalidated by poor, basic tissue culture practises.

The European Tissue Culture Society (ETCS) was formed in 1979 to promote the application of tissue culture to problems of cell biology and to provide an across-discipline forum within which scientists who use tissue culture could exchange information and experience. It was, and remains a particular aim of the Society to help young research scientists who need to acquire cell culture skills in order to conduct their research.

Most European countries have a national branch of the ETCS and the network is administered by a Council of members and a central Executive Committee. Details can be found on the main ETCS website **(www.etcs.info)**. Each national branch organises programmes of scientific meetings and every 2 years one member nation will organise an international meeting. All such meetings are publicised on the main website.

The UK branch of the ETCS (ETCS-UK) seeks to prosecute the aims of the ETCS mainly through organising or facilitating a programme of scientific meetings and workshops that focus on topical areas of cell science, or specialised applications of tissue culture. The York meeting (reported above) is an example. ECACC and Sigma support ETCS-UK and ECACC provides secretarial and administrative resources to the Branch. ECACC also dedicates pages of its own website **(www.ecacc.org.uk)** to ETCS-UK news and announcements.

ETCS-UK is run by a committee which is presently comprised of Professor Ian Mackenzie (Chairman), Dr David Lewis (Treasurer), Dr Caroline Wigley and Dr Angela Hague. Professor John Masters is President of the central European Executive Committee and also attends UK Branch Committee meetings.

Membership of ETCS-UK costs £20 per year (£10 to students), which entitles them to discounted attendance at all Society meetings. The discounts more than recoup the cost of membership. Members will receive newsletters both from ETCS-UK and the central organisation, together with early notification of forthcoming meetings. Income from membership fees goes towards administration costs and helps to underwrite the costs of meetings.

To apply for membership of the UK Branch, please email **lisa.reynolds@hpa.org.uk** and include your postal address so that we can send you an application form. To join any other national branch see the central ETCS website.

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