Serum-free cell culture – the ethical, scientific and economic choice

Focus on Alternatives is an organisation working towards alternatives to animal experiments. Here, Carol Newman looks at the advantages of serum-free cell culture

Cell culture technology is used in virtually all fields of biomedical research and testing. Since the early days of cell and tissue culture, animal serum has been added to the culture media as a source of nutrients and other ill-defined factors – a practice that exists to this day, despite technical disadvantages to its inclusion, its high cost, and the increasing availability of serum-free alternatives. Increasing concerns about animal suffering inflicted during serum collection add an ethical imperative to a move away from the use of serum wherever possible.

Collection of fetal calf serum

Serum can be obtained from a range of species, but the preferred source for cell culture is the calf fetus. Fetal calf serum (FCS) is prepared from blood extracted from fetuses removed from cows found to be pregnant at slaughter. The process and potential suffering involved has been explored in detail in a recent paper (*Alternatives to Laboratory Animals* 2002; **30**: 219-27). Briefly, the fetus is removed during evisceration, cleaned, the umbilical cord tied off and then blood is extracted via a needle inserted into the heart, without the benefit of anaesthesia.

In recent years there has been an increased awareness of fetal sensitivity to pain, and also a growing body of evidence that indicates resistance to anoxia in mammalian fetuses. Consequently, calf fetuses are likely to be alive and have normal brain function during blood collection, and may be expected to experience suffering until death occurs. It is estimated that one to two million bovine fetuses are subjected each year to this process, yet many scientists who use FCS regularly for cell culture remain unaware of the animal suffering involved in its collection.

Cell culture technology is used widely in biomedical science

Technical reasons for eliminating serum

Serum is a largely undefined, complex mixture of many and various constituents, some 200 of which have been identified so far. The effect of many of these on cultured cells remains unclear, and there is some evidence to suggest that there are cytotoxins in serum that have a detrimental effect on both primary and established cell lines.

Serum composition varies enormously, so laboratories need to screen each batch before use – a process that is both time-consuming and costly. Substantial batch-to-batch variation of serum makes it hard to control culture conditions, and different serum batches can produce different outcomes in cell-line assays. In addition, serum can habour contaminants such as viruses, bacteria, prions and mycoplasmas.

The high cost of FCS (top-quality FCS can cost up to £500 per litre) provides an economic incentive for the use of an alternative in cell culture, and fluctuations in serum availability have led to periodic crises in supply. One industry estimate attributes 55% of total material costs to the use of just a 5% serum concentration in culture medium.

Serum-free cell culture

Ideally, a defined and consistent medium is required for cell culture. In the past, researchers have encountered difficulties when attempting to culture cells without serum. Now, an increasing number of commercial companies supply serum-free media in which a wide range of different cell types can be cultured successfully without loss of viability.

Focus on Alternatives (FOA), an umbrella group of organisations promoting alternatives to animal experiments, has reviewed the availability of serum-free media for cell culture. A search of

the internet identified a total of 28 companies that supply a range of serum-free cell culture media, the details of some 200 of which have been compiled into a table of information (obtainable from carol@drhadwentrust.org.uk) indicating supplier, composition, applications and types of cells supported for each product.

Media that are entirely free of animal ingredients are highlighted, and an index enables researchers to easily locate the most suitable products for particular cell types.

The range of serum-free media available and the different cell types supported is extensive. Recent developments in this field appear to be driven by the large-scale production of biopharmaceuticals, where stringent purity requirements mean that serum-free cell culture conditions are essential.

A number of serum-free media have been designed and optimised to support specific cell types such as the commercially important cell lines CHO, Vero, MDCK and monoclonal antibody hybridomas. Other media are designed for general-purpose culture and can support a wide variety of different cells used in research.

Even media developed for a specific purpose may be more widely applicable, and some companies provide feedback on customers' successes in using media to culture cells other than those originally intended.

Content of serum-free media

Some commercial companies offer a serum 'replacement' that can be added to a culture medium in place of serum. However, the majority offer complete serum-free media, along with instructions on how to wean cells into serum-free culture conditions.

Most consist of a basal medium to which various compounds (amino acids, attachment factors, vitamins, growth factors etc) that perform known functions are added, and these may originate from animal, human, recombinant or plant sources.

By definition, serum-free medium lacks whole serum as an ingredient, but it may not be entirely free of serum-derived products. For example, the protein content of some serum-free media is bovine serum albumin.

Focus on Alternatives has not researched in detail the animal welfare implications of other animal-derived ingredients, but its primary concern is the extraction of blood from living fetuses. Products derived from adult animals, or at slaughter, may cause less suffering but media containing no animal-derived ingredients are preferable.

Many companies offer full details of the ingredients in their serum-free media directly to interested researchers, and a few list the ingredients on their websites or in technical product datasheets. However, as the production of serum-free media is a commercially competitive field, a few companies keep their patented media formulations a secret. This is not helpful to researchers.

Animal-free culture media

The purest and most consistent cell culture environment is a chemically defined medium entirely free of animal-derived components. Several companies now offer such specialised media and these are highlighted in the table of information prepared by FOA. Defined animal-free culture media are available to support various cell types including HeLa, breast cancer, kidney cells, CHO, stem cells, pancreatic islets, and hybridomas for monoclonal antibody production.

Using the purest possible nutritive environment is particularly important in the cell culture production of biopharmaceuticals for clinical use, and several companies cater for this requirement. Hyclone has a culture media manufacturing area maintained as 'animal derived component-free', and Gibco Invitrogen has a computerised database of raw materials that enables it to identify for customers any materials of animal origin.

An estimated one-third of biotechnology products currently under development are monoclonal antibodies. These are traditionally grown in media supplemented with relatively high concentrations of serum (5–20% v/v). However, several companies now sell specialised animal-free media for hybridomas, and these produce higher yields of monoclonal antibody and simpler downstream processing than were available with previous serum-supplemented media.

Weaning and performance

Persuading cells to grow in serum-free medium usually involves a gradual weaning process. Cambrex claims some cell populations can be converted rapidly to serum-free conditions by pelleting the cells and resuspending them in serum-free medium. In general, however, the purer low-protein or protein-free formulations require more attention during the weaning process.

Some companies offer a limited range of cells already adapted to their own serum-free media products. For example, Gibco Invitrogen supplies embryonic human kidney, CHO, COS-7L, primary human keratinocytes and various insect cell lines, adapted to serum-free media.

During weaning, it is necessary to monitor cellular function, as changes in culture conditions may affect the aspects of cellular function that the investigator is particularly interested in. However, the major benefits gained from switching to serum-free media can outweigh the effort required to overcome these initial hurdles.

Many companies claim that their serum-free media can outperform comparable serum-supplemented media, and publish literature displaying results for particular cell types that show comparable or better growth of cells, at higher densities and/or producing higher yields of endproduct.

Once cells have been adapted to serum-free media, researchers can benefit from improved control over culture conditions, the elimination of contaminant interference, improved reproducibility between cultures, consistency of media that avoids the need to screen batches, and avoidance of serum cytotoxicity.

An FCS-free future

Focus on Alternatives would like to see an end to the current widespread use of FCS and believes that the range of serum-free products already on the market, together with future developments in this area, hold great potential for the elimination of serum use in cell culture.

There is both a moral and legal imperative (European Directive 86/609/EEC) for scientists to use alternatives to animals, wherever possible. Already, cell culture has proved immensely valuable in replacing procedures on living animals; for example, cell culture production of polio vaccine has saved the lives of thousands of monkeys, and *in vitro* production of monoclonal antibodies has largely replaced a painful method of raising them in the abdomens of living mice. Clearly, the value of cell culture methods to animal welfare would be further enhanced by the removal of serum, in particular FCS, from culture media.

Economic and safety reasons have provided the impetus for industry to switch to serum-free culture conditions for biopharmaceutical production, and have paved the way for biomedical researchers to follow suit.

Weaning cells off serum-supplemented medium and into serum-free conditions may cost researchers some time and effort, but this investment would be repaid by the consistency and quality of results. FOA would urge all users of cell culture to make the extra effort to switch to serum-free media. ■

FOA welcomes feedback on ways of encouraging and extending the use of serum-free media, and may be contacted through carol@drhadwentrust.org.uk or at www.focusonalternatives.org.uk.

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