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Factors limiting the commercial application of animal cells in culture

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Abstract

The application of quality control and assurance procedures to the components of animal cell cultures has transformed what was an art to a viable industrial technology. This results in the successful large scale operation of such cultures. However it is clear that the cost of obtaining a license to produce materials from animal cells in culture severely impedes the movement of products into the market place. It is therefore necessary to examine in more detail the reasons for the reluctance of the regulatory authorities to issue product licences and in particular to appreciate the way ethical issues influence this process. This paper reviews these issues and indicates a way ahead.

Introduction

Whereas the traditional view of animal cell biotechnology was that it was an expensive, unreliable and difficult technology, recent developments have dispelled these moribund perceptions. Under the appropriate conditions which are conductive to growth, animal cells in culture are robust and are not liable to shear disruption by the mixing or bubble aeration systems. Hiroki Murakami, his colleagues and friends made many contributions to this state of affairs and it is a tribute to him and them that what was once considered a "black art" requiring "green fingers" is now a set of procedures in practice in hundreds of commercial establishments, academic departments and research institutes in most of the countries of the world.

These contentions have been given added weighting in recent years. C. Thomas and Al-Rubei M. *et al*. (1994) have measured the forces needed to break a cell by compressing it in an 'anvil' type arrangement while under continuous microscopic examination. They compare this to the physically compressive forces likely to be obtained in a stirred tank and find that stirrer speeds and diameters which are well outside the range of conditions generally used for the mixing of animal cell cultures need to be generated before cells enter the zone where damage could occur. These observations concur with the theoretical approach based on the comparison of the Kolmogaroff length in turbulent fluids to the diameters of the cell. This dimension is dependent on the power input per unit volume of culture and the viscosity of the fluids. Again stirrer speeds and diameters which are respectively higher and longer than those necessary for mixing the cultures need to be generated before the Kolmogaroff length becomes of comparable size to the diameter of the cells, at which point damage can be expected. This situation does not pertain to quite the same extent in microcarrier cultures. Here cells are attached to particles of 200 micron diameter beads. At this scale, the dimension of the whirls in the turbulent culture medium may be similar to or smaller than the microcarriers. Under such circumstances there is a decrease in the cell productivity of the cultures. Cherry and Papoutsakis, (1990). Nevertheless, in neither of the two cases discussed do the mixing conditions have to be so vigorous necessitating the generation of cell damaging effects. Rather it has to be restated that the mixing conditions are defined in terms of distributing the cells homogeneously throughout the bioreactor and preventing cells and/or microcarriers settling on the bottom of the reactor. Such a duty can be performed by a low shear 'marine propeller'. This is unlike other microbial cultures where the mixing systems has the dual role of evenly distrib-

uting the cells as well as breaking down the size of the gas bubbles as they emerge from the sparger to enhance the oxygen transfer rates by increasing the bubble surface area. In addition, the appropriate formulation of the culture medium to include the Polyol Pluronic F68 provides a further level of protection and prevents cell degradation even in stirrer conditions which may in the first instance be considered to be capable of generating cell damaging environments (Cherry and Papoutsakis 1990).

The aeration of cell cultures has received much attention by this author and his coworkers ((Spier and Whiteside (1983), Spier and Whiteside (1990) and Spier (1996), Handa-Corrigan (1990) and Guderman and Lutkemeyer (1994)). Again theoretical calculations based on values of oxygen transfer coefficients of fully formulated culture media by Spier and Whiteside (1990) have shown that it is possible to provide rapidly respiring cells with enough oxygen at cell concentrations which are half of those present in normal animal tissues (approximately 10^9 cells/ml). Such a rate of delivery of oxygen depends on the transfer rate and the surface area available for that transfer. The latter can be augmented by using spargers and the Polyol Pluronic F68 which recent work (Zhang and Handa-Corrigan (1992)) has shown to be responsible for the generation of bubbles of less than 0.1 millimetres in diameter. This increase in surface area is only in part countered by a decrease in the oxygen transfer rate in the presence of such Polyols such that the overall transfer of oxygen is yet sufficient for cells in high concentration cultures. Of course there is a tendency for high concentration cell cultures to respire at a lower rate than maximal in which case the oxygen delivery conditions will cope with the required oxygen demands. Further theoretical computations (McCullough and Spier, 1990) have shown that it is possible to provide oxygen to the centre of a cell clump if that clump is about 0.5–0.75 mm in diameter. This dimension then becomes a critical variable when designing hollow fibre bioreactors, porous microcarriers and setting the conditions for aggregate based cell cultures.

Implementation of the necessary upstream control procedures have all but eliminated contamination problems in commercial installations which are generally admitted to be about 2% per annum. Additionally, the expence and unreproducibility of the cultures has been overcome by the use of serum or protein free medium. This change has been ushered into the main stream of animal cell technology by the need to produce products which are free of viral and prion contamination. As the most likely source of such contamination is held to be the animal sera and the complex undefined components of the medium the derivation of media with the appropriate growth factors and nutrients has been of crucial significance in overcoming this obstacle to commercializing processes.

By the thorough pretesting of the cells and media coupled with the use of high cell concentration systems to improve process intensity it has become possible to mount the successful operation of many large scale processes such as those which generate Namalwa Interferon, Polio Vaccines, Erythropoietin, t-Plasminogen Activator and Monoclonal Antibodies. Such processes are able to complete on economic terms with the production of similar materials from bacteria or yeast based systems. The evolutionary increases in the yields of monoclonal antibodies, to over 1 gm/litre in animal cell cultures, (Birch *et al*., 1996a) coupled with the ease of concentrating and purifying the product, as it is both secreted into a medium which has few large molecular weight materials to interfere with the downstream operations, has led to process improvements which belie the contention that the production of animal cell biologicals is an expensive way to proceed for a commercial product.

The regulatory hurdles

Nevertheless, the regulatory hurdles which required an average $230 million per product in 1991 (Vagelos, 1991) now require some $360 million and 10 to 12 years of testing and documenting to surmount the regulatory agency hurdle although more recently the time taken to obtain a license has decreased (Kessler and Feiden, 1995). Such considerations continue to dominate the technologies which are used and the commercial decisions that are taken when selecting particular products and the effort which will be deployed in obtaining the licence to produce and sell. In a recent issue of Scientific American, (Kessler and Feiden, 1995) it is argued that it may be possible to begin to market drugs (and by analogy, biologicals) before the final approval of the regulatory agency has been obtained. Indeed such pre-licence marketing can become part of the testing system required by the agency.

The magnitude of these regulatory requirements is determined by the need do demonstrate safety, efficacy and the consistency of the production process. While the last issue is incapable of resolution by the achieve-

ment of the necessary technical competence (some 1000 pages of documentation are required for each-batch produced during the 3 batch validation procedure and thereafter, (Birch *et al.*, 1996b) the two former concerns cannot be resolved merely by presenting the appropriate data. For such reasons manufacturerers are impelled by the 'successful' documentation they have amassed in-house to keep to the processes for which they have already had approval.

Assessment of values

Thus, with regard to safety and efficacy there has to be an assessment if the cost/benefit ratio, for it has to be understood that it is not possible to assert that any product is 100% safe. In establishing an estimate of this ratio there are major uncertainties as to the degree of cost as well as difficulties in assessing benefits. The costs are not just dependent on the materials, labour and capital costs incurred in the production of the biological. Rather there has to be an assessment of the possible damage to people. This can result in death or in the debilitation of individuals. Such costs are not generally accrued to the same evaluation scales which are used in the materials and labour situations. We can of course turn to the courts where judgements are made in monetary terms for the death or debilitation of individuals which arise from car crashes or building site accidents. This is however a less than perfect system. Individual courts differ considerably on the awards of damages and sometimes law specifically states the level of damages. Again such figures are modulated by the extent to which the court considers the affected individual to have been responsible for his/her actions and the corresponding outcome. Different juries in different jurisdictions also are capable of awarding wildly divergent damages in such cases. While this may put a monetary cost on such a loss there are consequential losses which are not brought into the equation. A key person in an enterprise may be so pivotal that the whole enterprise may cease to survive without that individual, spouses and families may perform at less than what they would have been capable of, leading to divorces, career upsets and often financial ruin (this often resulting from taking action though the courts to obtain recompense). The economic consequences or value changes which result from the elimination of a feel-good factor in particular circumstances bedevils the 'objective' evaluation of the economic equivalent of the loss of a dear one or the confinement of that individual to a crippled condition. On the other hand the probability of incurring such a loss can be determined with some degree of accuracy from animal and human testings coupled with a knowledge of the relevant theoretical mechanisms which underlie the use of a particular biological. Thus the estimation of the magnitude of the 'down-side' of a biological product is difficult; is the estimation of the benefit any less fraught?

Simple benefit considerations can be obtained from estimates of days in hospital foregone, doctors bills not delivered, drugs not purchased and days a work not lost. Such is the easy side. When someone is not stricken much more is at stake than the above list of benefits. There are the intangible changes in attitude which results from a well feeling; the optimism which leads to great enterprise and investment in the future, the communication to others that the positive side should receive greater consideration, that risks are worth taking and above all there is a sense that one can achieve what one sets out to do, (one seems to have more of the 'right stuff' (Wolfe, 1979)). The added productivity of the worker (what is the value of the added productivity of the mother looking after a family on a full time basis?) can be calculated but this is a minuscule benefit compared to the added value that a 'feel-good' attitude can deliver when translated into economical terms. In short it is not possible to adequately quantify benefit at this time (more research is needed here) so that the regulatory agencies are left with a conundrum: unable to sensibly assess the cost/benefit ratio they have to rely on other factors in making decisions on when they have seen enough documented evidence of the safety and efficacy of a proposed product; one such factor is their sense of ethicality.

Ethical considerations

Ethics is our knowledge of how to behave in a correct fashion. Sometimes we are pressurised by our immediate and distant peers into behaving in a particular way. The regulators populating the regulatory agencies are subjected to just such pressures. The rapid licensing of the drug AZT as a putative treatment for AIDS patients is one such case. On the other hand the experience in Europe of the depredations of the improperly licensed drug Thalidomide have made regulators restrictive, conservative and unwilling to accept the novel or unfamiliar. Such a behaviour while having an ethical dimension in that a behaviour is being

determined by such considerations, the latter also have psychological determinants.

The acceptability of the foreign or new requires thought processes which have to overcome innate resistances. The basis for such resistance may be seen in two analogous situations: the performance of the immune system and the way societies work in relation to foreign societies. The immune system responds most violently to situations in which it is neither completely overwhelmed nor barely stimulated. We can readily identify immune exhaustion and low dose tolerance with these two conditions in which the immune system does not reject the invading foreign material. Similarly, the presence of a few foreigners in the midst of a large society goes unnoticed except perhaps as an interesting curiosity; the foreigners are generally not rejected. Also when the foreigners are an overwhelming majority they become accepted as a matter of course for to do otherwise would be counterproductive. Can it be that the minds and thought processes of the regulators (and for that matter, ourselves) respond in a similar way? Novel ideas are the ones which are most often rejected; (consider the rejection of novel papers by Nature and the Royal Society) if they are fed to us in small inconsequential doses we may take them on board, failing that we have to be beset with an overdose of material which makes the case overwhelming (at least it shows that we have 'done the due diligence') (Spier, 1996b).

In addition to the psychological factors which condition the way we behave there are other influences. The regulator is a member of a society. The regulations which the regulator works to are generated by the governmental organs of that society. Both the regulators and the governments are subject to opinions and attidues which prevail in the society. Such ideas are carried by the press and media in all their multifarious manifestations. Often the key issues are based upon the guidelines which are laid down as social ethics. These may derive from religious institutions or from secular sources. In contemporary societies the former are pluralistic, which both creates problems, for the religious institutions do not speak with one voice, but it also creates liberties for other inputs from secular sources. Needless to say the ethical input to the regulators, whether direct or through government is not coherent. The determination of benefit, is it more people or a higher quality of life? is left indeterminate. The regulators are thus left bereft of clear guidelines as to how to deal with the issue of benefit. However when new kinds of products arise they generally respond by presenting the proposing commercial concern with hurdles so high as to be virtually impassable. For example, the materials Erythropoietin and Human Growth Hormone can be used to ameliorate human conditions which are not diseased. Will the regulatory agency sanction (license) the use of such materials where there are not clear indications of preexisting distress? Other such materials are in the pipeline.

Conclusions and the way forward

It is clear therefore that technical issues are not crucial to the development and promulgation of products made from animal cells in culture. Rather the perception of the safety and ethical acceptance of such materials is becoming increasingly important as a determinant of the commercialization of such products. There are many issues which have to be taken forward to give the animal cell technologist a clear view of the kinds of products which society will be demanding in the decades ahead. Firstly there has to be a clear shift in the proportion of our efforts which are spent on therapeutics and an commensurately increased effort on prophylactics. The latter may be most effectively delivered through the encouragement of disease sparing ways of eating, exercising, thinking and controlling the quality of our environment; but then we have to consider protecting ourselves by preconditioning our immune systems and by changing our genetic makeups so that we do not fall to diseases which can be predicted to happen from a reading of our individual genomes (in selected locations). We will also have to provide the society at large with the tools to achieve a population level which is appropriate for sustainability. These latter tasks have not yet reached the desk of our regulators. But when they do we may hope that will have the appropriate ethical armamentarium to deal with them on all our behalfs.

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