AICHE

DIFFERENT PERSPECTIVES ON CELL CULTURE

NEW YORK—When chemical engineers cogitate about mammalian cell culture, they are more apt to think in terms of fluid dynamics and shear forces within a bioreactor than they are to consider expression vectors and levels of protein production.

Their perspective has validity, because the decreased growth rate and cell death that can occur in cultures grown on microcarrier beads may often be due to mechano-physical forces rather than metabolic problems. According to Robert Cherry (Rice University, Houston, TX), speaking at the American Institute of Chemical Engineers meeting held here in November, microcarrier beads can get caught up in the reactor's impeller-generated turbulence eddies. The beads can also collide with each other, depending on the eddies' velocity.

The answer to this problem is *not* decreased agitation rates, however. Cherry and his associates have found that low agitation rates encourage bead-bead bridging, even with beads that do not contain cells. Under these conditions, the growth rate of bovine embryonic kidney cells decreases. The larger particles formed by bead clumping can often lead to cell damage, as well. Conversely, if the scientists grow the cells on smaller beads, their growth rate increases, as it does in higher viscosity medium.

Another current favorite method of culturing mammalian cells is to grow them in hollow-fiber bioreactors. Whereas biotech companies use these reactors to economically produce recombinant proteins or monoclonal antibodies, these systems are also useful for nuclear magnetic resonance (NMR) studies of living cells. NMR has several advantages, the main one being that it is non-invasive; cells can be studied without destroying them. Because NMR can distinguish between different atoms—³¹P and ¹³C being the most frequent targets—the method can be used to measure intracellular pH, bioreaction rates, and transport rates, among others. But the technique is not very sensitive: If there is not an abundance of molecules in the sample, the measurement takes too long.

To address these drawbacks, Bruce Dale and collaborators at Colorado State University (Fort Collins) have designed a hollow-fiber bioreactor specially for NMR studies. Because the scientists wanted to be able to determine the actual cell density inside the reactor, they also devised a

technique to measure cell number on-line. Current methods—such as monitoring glucose uptake and correlating that value with cell density—are largely inferential. Dale's technique instead measures the electrical conductivity of a whole cell suspension.

To configure the electrode within the reactor, the scientists laid out several hundred very thin hollow fibers in parallel. At each end, they placed a conductivity probe wire on top of and perpendicular to the fibers. When the whole assembly is rolled up on a spool, the resulting bundle of fibers contains the electrode coiled inside, nestled between the fibers in the extracapillary space (where the cells also reside).

The goal, says Dale, is to maintain maximum cell density under defined conditions. In fact, the cell density these reactors attain is about 30 per-

cent of the reactor volume. (Typical cell densities in commercially available hollow fiber bioreactors run at about 10 percent, he adds). The maximum local cell densities in these bioreactors, as measured by the new conductivity probe, is about 2×10^8 cells/ml. Dale's research has also determined that cell growth in the fiber bundle is not uniform; it varies axially.

Although it is possible to measure cell density via electrical conductivity, Dale warns that the Ehrlich ascites cells used in these studies are not perfect insulators, as had been thought. They have a substantial electrical conductivity of their own, which must be taken into account. What effect a secreted cellular product would have on conductivity measurements has not been determined.

—Jennifer Van Brunt

RETROVIRAL VECTORS

COMMERCIALIZING GENE THERAPY

NEW YORK—Both Viagene Inc. and Genetic Therapy Inc. are trying to turn molecular biology's dream of gene therapy into commercial reality. Their common goal is lofty: to use retroviral vectors to deliver genes into selected cells within the body, and then direct that genetic material to integrate into the host's genome and express appropriately. Thus, in theory, a patient's own cells could be jury-rigged to produce key enzymes or other proteins needed to correct his or her disease.

Viagene (San Diego, CA), formed in February 1986 by Gensia Pharmaceuticals (San Diego, CA), will use gene transfer to treat viral infections, malignancies, and genetic diseases. Born out of Theodore Friedmann's pioneering research at the University of California (San Diego), the firm will initially address relatively nearer-term therapeutic opportunities, such as fighting cancer and AIDS. In cancer therapy, for example, products made by retrovirus-delivered genes could act as markers to aid in the targeting of other therapeutic agents. Similarly, transferred genes could be used to produce protein antigens that would act as vaccines.

This past December, Viagene raised an initial \$1 million from a group of venture investors that included Biotechnology Investments Ltd., Domain Partners, 3i Ventures, and Fairfield Venture Partners.

The other gene-therapy upstart, Genetic Therapy Inc. (GTI, Gaithers-

burg, MD), will be collaborating with W. French Anderson at the National Heart, Lung, and Blood Institute of the National Institutes of Health (NIH, Bethesda, MD). The year-old company has already sponsored work in academic laboratories and is currently renovating a building in Gaithersburg to provide some 8,000 square feet of lab space. According to GTI president M. James Barrett, formerly president of Life Technologies Inc. (Gaithersburg, MD), GTI is operating with \$2.5 million provided by Steinberg and Lyman Healthcare Ventures.

Anderson, who may not have a financial stake in GTI under NIH rules, stresses that he is developing basic gene delivery technology that will come to fruition perhaps a decade from now. Much of his work has focused on adenosine deaminase deficiency. "That's a good model," says Barrett, "although in the long run it is probably not a good commercial opportunity." GTI would be more likely to address a condition like factor VIII deficiency in hemophiliacs, he says. And opportunities exist in the veterinary field as well, such as increasing the expression of bovine growth hormone in cows.

Anderson, however, emphasizes that it is too early to discuss commercialization: "There is no sense talking about what the first cargo in the space shuttle will be until you develop the space shuttle's engines."

—Arthur Klausner