SORTING CELLS FOR MEDICINE

The basic technology for cell separation hasn’t changed much over the past several years. What has changed dramatically is what those cells are being used for: treating patients. As the promise of cell therapy is gradually realized, researchers are searching for cell separation technologies that can solve some basic problems, such as improving cell processing speed and making processing as sterile as possible. Researchers already use a mix of density-gradient separation, magnetic separation, and flow cytometry to prep cells for clinical use but hope for newer chip-based technologies to help advance the field. By Anne Harding

Cell therapy has technically been around for decades—think bone marrow transplants—but now researchers are finding evermore sophisticated ways to manipulate and use cells for medicine. Physicians and scientists are harvesting dendritic cells, T cells, stem cells, and more from patients’ bodies and identifying and separating those with the strongest potential for helping patients. Once isolated, these cells can be grown in large quantity and returned to a patient’s body to treat various diseases.

Though cell therapy remains a lively area of research, commercially available treatments have been slow to enter the clinic. The U.S. Food and Drug Administration (FDA) approved the first-ever autologous cell therapy (which manipulates the patient’s own cells and returns them to his or her body), Genzyme’s Carticel, for treating damaged knee cartilage in 2007. The second, Provenge, for treating metastatic prostate cancer, followed in 2010. Last March, the Centers for Medicare and Medicaid Services announced that it plans to cover this $93,000 prostate treatment, a major boost for Dendreon, the Seattle-based company that makes the drug.

Allogeneic cell therapies, made from cells harvested from donors rather than the patient, offer the possibility of “off the shelf” use. Dozens of candidates are currently in clinical trials for applications ranging from boosting wound healing to treating graft-versus-host disease to supplementing blood cancer chemotherapy treatments.

Because these cells are being used for medicinal purposes, the FDA regulates the entire process under the same rules that govern the production of pharmaceuticals, known as good manufacturing practice (GMP). Given the vast potential for cell therapy, many companies making instruments, reagents, and other tools for cell separation have shifted their focus away from the research lab and into the translational and clinical research space.

But researchers in the field say many gaps remain in what’s available to develop clinically viable cell therapies, from instruments to antibodies. And because the payoffs so far have been relatively few and far between, some companies making these instruments or reagents have had to pull their products off the market, such as with Baxter’s Isolex 300 Magnetic Cell Selection System—leaving researchers who use their products high and dry, especially those with open clinical trials under way.

“It’s really hard to depend on a single manufacturer,” says Lynn O’Donnell, who directs the Cell Therapy Laboratory at the Ohio State University’s James Cancer Hospital in Columbus. “Given the rigid regulatory environment, the manufacturers in this clinical space need to have a long-term outlook and a commitment to cellular therapy and regenerative medicine.”

BACK TO BASICS: CELLS OUT OF TISSUE

Separating cells from their tissue of origin is one of the real bottlenecks for developing cell therapies for clinical use, says Firman Ghouze, director for cell therapy within GE Healthcare Life Sciences. While sorting cells out of blood is fairly easy, isolating them from fat—which has turned out to be a very rich source of stem cells—is another matter.

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Companies are putting a lot of effort towards developing simpler high volume, high throughput processes for separating cells from tissue that can be handled by technicians and not only Ph.D.s, according to Ghouze.

To this end, GE offers the StemSource 900/MB Tissue Processing System, which was developed by Cytori Therapeutics. This sterile, closed centrifugation system can be used for extracting several different regenerative cell types from connective tissue, yielding an average of 4.6 x 10^7 nucleated cells per 100 grams of tissue. GE’s Res-Q 60 Bone Marrow Concentration System, also centrifuge-based, can be used at the patient’s bedside to isolate mononuclear cells from bone marrow in less than 20 minutes.

O’Donnell and her colleagues separate islet cells out of pancreatic tissue using another popular centrifuge-based instrument, CaridianBCT’s COBE 2991 Cell Processor. The instrument uses a closed fluid path and single-use disposable set for cell centrifugation—a vast improvement over traditional centrifuges and conical tubes because it allows various fractions to be collected within a closed system, O’Donnell explains.

But the density gradient system has limitations when it comes to coping with pancreatic islets, which “are kind of like mini-organs because they are clusters of cells,” she adds. The instrument is nonspecific and variable between islet isolations.

“It’s challenging to come up with a purification technique that works for large clusters of cells and variably sized cells,” O’Donnell says. And given the nature of islets, “you end up sacrificing yield in order to gain purity because the separation isn’t great.”

CLOSED SYSTEMS ARE KEY FOR CLINICAL PRODUCTS

Most researchers in the field, including O’Donnell, use a combination of centrifugation, magnetic bead separation, and flow cytometry to develop their cell therapies. Magnetic separation has the advantage of speed, while cell sorting allows for the use of multiple markers and results in a purer end product.

“In the case of magnetic bead systems, a whole new series of reagents and equipment have been developed that allow for more automated processing and more closed operation, so you can get a sterile cell product,” says Shelly Heimfeld, who directs the Cellular Therapy Laboratory and cGMP Cell Processing Facility at the Fred Hutchinson Cancer Research Center in Seattle. Heimfeld is also past president of the International Society for Cellular Therapy, an organization that supports the translation of cell therapy research into clinical use.

Such instruments include Miltenyi Biotec’s CliniMACS platform, which uses magnetic-based technology for enriching target cells or depleting unwanted cells. Miltenyi is about to introduce a second-generation version of the instrument, the CliniMACS Prodigy. “The idea is to have the entire GMP-conformed process for cell manufacturing integrated into one device,” explains Jurgen Schmitz, head of research and development at the Bergisch-Gladbach, Germany-based company. This closed system can wash, separate (with density gradient cell separation or magnetic bead cell separation), and formulate the cells of interest, Schmitz explains.

“That is something quite complex in terms of logistics,” he adds. “Such an instrument can really help make things easier and less expensive, for example by reducing clean-room requirements.”

Because companies are focused on making cell-sorting instrumentation and reagents that are suitable for use in clinical products, they are now “thinking about how to comply with GMP-manufacturing guidelines,” says Tim Fong, technical director of cell therapy at BD Biosciences.

Maintaining the sterility of cell products is key. BD Biosciences, for example, offers a replaceable gamma-irradiated fluidics kit for the Influx cell-sorting platform. “All the tubing that the cell sample comes into contact with on the instrument is single use and gamma irradiated, which mitigates the prospect of serial contamination,” explains Jack Dunne, who leads the company’s cell-therapy research team.

ACCELERATING CELL SORTING WITH MULTI-MICROFLUIDICS

The ideal technology for producing cell therapeutics, says O’Donnell, would be a speedier version of flow cytometry since “you can look at multiple markers and purify very specific kinds of cells.” But, she points out, there’s the challenge of engineering a single-use fluid path that allows cell sorting under sterile conditions. One solution, she says, is to put a box around a standard flow sorter that would provide clean, HEPA-filtered air, “but you still have cross-contamination questions, like what do you do between processing for patient A and patient B.”

Another possibility researchers foresee is the use of microfluidics chips that sort cells via microscopic channels and gates that open and close to determine the cells’ flow path. With several flow paths running in parallel on a single chip, the sorting process could be sped up. 

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considerably. What’s more, the system could be entirely closed and aseptic. Feasibility studies by developers, including Owl Biomedical, have been promising, according to David DiGiusto, who directs the Laboratory for Cellular Medicine at the Beckman Research Institute of the City of Hope in Duarte, California.

Yet another advantage of electrostatic cell sorting on chips is that it would be “label-free.” While centrifugation techniques are label-free also, both magnetic cell separation and flow cytometry require tagging cells of interest with antibodies or dyes, creating another challenge for researchers: finding ways to remove the labels such as using enzymes that can “clip off” antibodies after sorting or designing biodegradable labels that simply fall apart. “We’re still in an era where we’re using the [same] methods that identify the cells to mediate their separation,” says Adrian Gee, a professor in the Department of Cell and Gene Therapy at the Texas Children’s Hospital.

EVADING REJECTION TO MAKE ‘OFF-THE-SHELF’ PRODUCTS

So far, large pharmaceutical companies seem to have held back from getting involved in cell therapeutics, which remains pretty much the province of startup biotechs and translational research institutions.

“It’s much more attractive for companies to have a generalized cell product that could be used to treat a wide variety of patients,” says Gee. But developing such generalized products requires figuring out a way to ensure that the recipient’s immune system accepts the donated cells. Just as with blood transfusions and organ donations, cell therapies can be rejected by a patient’s body. Solving this problem may, in some cases, require using tissue matching techniques like those used for blood donation or organ transplantation.

Investigators have also learned that some cells, such as mesenchymal stromal cells and nerve cells, do not provoke the rejection responses seen with other cell types. Gee and his colleagues found that T cells primed to attack viruses can circumvent human leukocyte antigen (HLA) barriers, the immune-system “tissue types” that must match between donor and recipient to prevent rejection. “We used to think that these [T cells] could only be used in essentially closely matched tissue recipients, but it appears they can be used across some tissue types;” he explained.

LOTS OF PROMISE, BUT SLOW PROGRESS TO THE CLINIC

“There’s a lot of things going on” in the development of cellular therapies, but progress is “slow and painful,” says Robert S. Negrin, a professor of medicine at Stanford University, director of the university’s Bone Marrow Transplant Program, and medical director for the Stanford Cell Therapeutics Laboratory.

One factor delaying things, explains Negrin, is the scarcity of antibodies that are made to GMP standards for magnetic cell separation. He estimates that there are currently a dozen or so antibodies, made by BD, Miltenyi, and other companies, that are suitable for preparing cells for clinical use. “That’s where we often get stuck, is having the proper reagents,” Negrin says. Just having more antibodies available for clinical use could give the field a major push forward, he adds. Yet, as with many other technologies related to the field, investors and companies are shying away from such investments until they see some real success.

However, investments in products that support cell therapy research is just as essential as the research itself, says DiGiusto, since “one without the other does no good.”

Despite the challenges of developing commercially available cell therapies, more than a dozen companies have allogeneic or autologous products in various stages of clinical trials, including Geron, Osiris Therapeutics, Cytori Therapeutics, and Aastrom Biosciences.

And with each success story, such as Dendreon’s prostate cancer drug therapy, Provenge, the field as a whole benefits, explains DiGiusto. “It’s at least a sign to the investment community that [success] can happen,” he adds. “And it’s really a critical thing that the investment community continues to have confidence in the field.”

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