Researchers mass-producing stem cells to satisfy the demands of regenerative medicine

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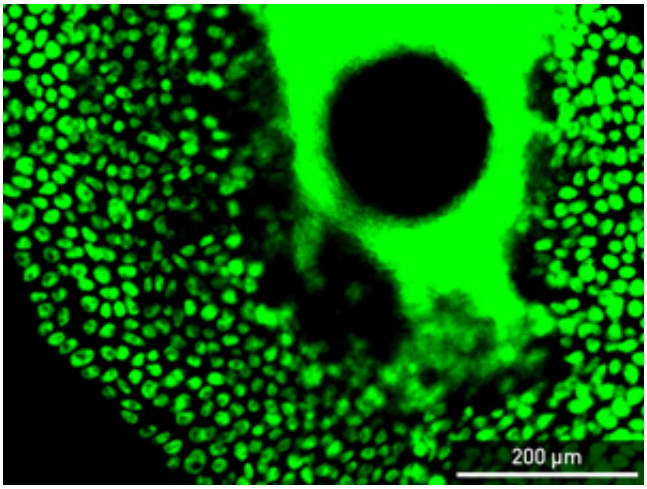

Human embryonic stems cells (green) aggregate around spherical microcarriers (small black circle in center) floating in a nutritional brew, a technique that can be used to grow millions of stem cells. Credit: A*STAR Bioprocessing Technology Institute

Steve Oh had been growing stem cells by conventional means at the A*STAR Bioprocessing Technology Institute (BTI) for seven years, when in 2008 his colleague Shaul Reuveny proposed an idea for speeding up the process.

Instead of culturing the cells on round, flat Petri dishes, he could try growing them on tiny polystyrene beads known as microcarriers floating in a nutritional brew, suggested Reuveny, a visiting scientist at the BTI.

This technique had been used for decades to mass-produce virus-infected cells for the vaccine industry, which Reuveny was very familiar with.

The average Petri dish fits fewer than 100,000 cells, a miniscule amount when stacked against the 2 billion muscle cells that make up the heart or the 100 billion red blood cells needed to fill a bag of blood. The approach Reuveny suggested potentially could produce cells in much vaster numbers to make them more practical for therapy.

"Why don't I bring some of these microcarriers over to you?" Reuveny suggested to Oh. Eventually, Oh was convinced to try what could become the most scalable method for growing stem cells and differentiated cells worldwide.

"There is a trend now in industry to move away from this simple Petri-dish method to manufacturing stem cells in bioreactor processors," Oh says. "We started this journey much earlier than everyone else in the world."

Exponential growth

Oh's Stem Cell Group first tried the approach on human embryonic stem cells. These are found in the early embryo and have the potential to mature into any type of cell in the body, a state known as pluripotency. For months, they struggled to develop a coating that would make the stem cells stick to the microcarriers, and to formulate a solution that contained the right mixture of nutrients for the cells to grow. "Without Reuveny's know-how, we probably would have failed," says Oh.

About a year into their experimentation, one line of human embryonic stem cells survived past the 20-week mark of stability. Not only were these cells viable, they were also two to four times more densely packed

than those grown in Petri dishes.

The group has spent the last six years refining their processes to produce even more cells using cheaper materials and fewer steps. "We are easily achieving three times higher cell densities than the Petri dish approach," says Oh. "In some cases, by modifying the feeding strategy, we can get six times more cell densities, and we could probably reach ten with a bit of work."

The process can be scaled up exponentially in larger tanks. "If in one week we can go from a ten-milliliter culture volume to a hundred-milliliter bioreactor, then the next week we can go from a hundred milliliters to one liter; and ten liters the week after that," explains Oh. The equivalent in Petri dishes—from 100 to 1,000 to 10,000—would be practically impossible for a researcher to handle.

The team has also expanded their repertoire to two other types of stem cells—induced pluripotent stem cells and adult mesenchymal stem cells—as well as differentiated heart, neural, bone and red blood cells.

Healing hearts

The biggest advances for Oh's team in recent years have been in the growth of differentiated heart muscle cells, called cardiomyocytes. "We beat the Petri dish method on all counts—purity, yield, cost of goods and simplicity of process," he maintains.

But a lot of their success is thanks to protocols initially developed on Petri dishes. For a start, cardiomyocytes are the fastest cell type to differentiate, taking only two weeks. And researchers have developed a method to grow pure batches of cardiomyocytes without the addition of expensive growth factors. Instead, they use small molecules to first inhibit and then activate a key cell-differentiation pathway known as

Wnt signaling. Oh's team applied this small-molecule approach to grow and differentiate cardiomyocytes from embryonic stem cells directly on their microcarriers.

"We can hit 90 per cent cardiomyocyte purity with this activator–inhibitor protocol," says Oh, and the process is five to ten times cheaper than the Petri dish approach. Other preliminary results from their bioreactors reveal densities of millions of cells per milliliter, nearing the dosage counts needed to use stem cells in regenerative medicine.

The ultimate goal of the research is to grow enough cells in an affordable way to patch up one square-centimeter of damaged heart muscle following a heart attack.

Oh's team is now partnering with industry to further improve the process, as well as with clinicians to test the healing potential of their cells on animal models. "We always try to keep the end in mind, to translate our work into something that can eventually be used by companies and clinicians."

Healing tissue

Heart cells are just one cell type being grown at the BTI. From embryonic stem cells, the team has also developed progenitor cells halfway to becoming mature neurons, as well as dopaminergic neurons that when progressively lost in the brain can cause Parkinson's disease. And the team is in the early stages of differentiating red blood cells at speeds and scales sufficient for use in emergency blood transfusions.

From mesenchymal cells, for which there are currently more than 400 registered clinical trials worldwide, Oh's group is growing bone and cartilage cells known as osteoblasts and chondrocytes that can be

introduced to animal models to repair damaged tissue.

"There is a need for better clinical products to heal bone defects and fractures or to treat those in need of spinal fusions. Stem cell therapy shows promise in this area," says Asha Shekaran, a biomaterials and cell therapy researcher at the BTI. Asha investigates the bone-forming and bone-healing potential of stem cells grown on biodegradable microcarriers by implanting the cells just under the fat in the back of mice and delivering them into skull defects in rats.

"One of the challenges with evaluating stem cell treatments in complex living systems is that there is often a large degree of variability," says Asha. "The results don't always translate very well from in vitro to mineralization in a non-bone site to bone healing in a rat model. And it is even harder to say how it will fare in larger animal models and clinical trials."

But her preliminary results on the ability of scalably grown stem cells to differentiate into osteoblasts and produce cell-signaling cytokine molecules have been encouraging, she says. "Over the next five years, there are going to be a lot of strides made in translating stem-cell-based products into commercial use, especially in the scaled-up manufacturing of these products."

Thanks to their pioneering use of microcarriers for a wide variety of differentiated cells, Steve Oh and the Stem Cell Group are well-positioned to be part of that exciting new future.

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