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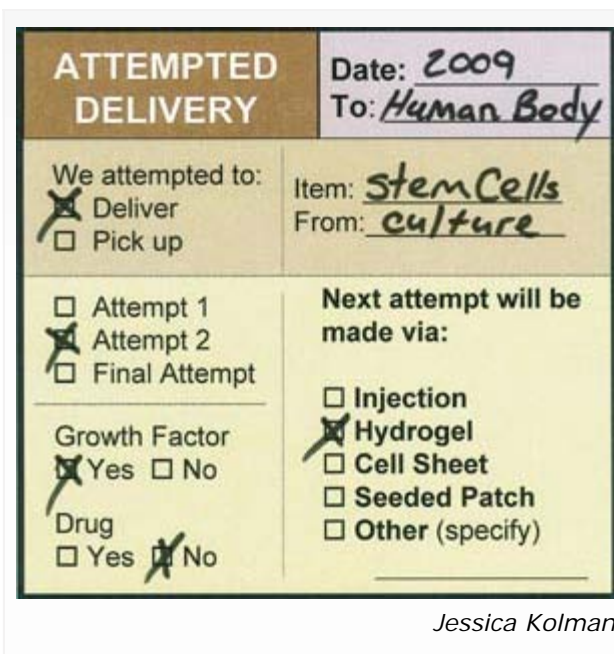
## The delivery dilemma

Megan Scudellari<sup>1</sup>

**If stem cell therapies are going to succeed in the clinic, researchers must determine the safest and most efficient ways to transplant cells into the body.**

Swaddled from head to toe in a sterile white suit, a technician at Geron Corp. pads across the clean room to an incubator and carefully lifts a sloshing tray the size of a laptop. The human embryonic stem cells (hESCs) growing in the culture will soon to be differentiated into oligodendrocyte progenitor cells, to be used later this year in the first clinical trial of a hESC-derived therapy to be approved by the US Food and Drug Administration (FDA). But turning the cells into oligodendrocyte progenitors may prove much easier than moving them into people.

Companies approaching clinical trials are racing towards a hurdle that cannot be cleared by laboratory research alone: what is the best way to get cells into the human body? Traditional injection methods, popular with animal models, often result in poor cell survival and low levels of cell integration into host tissue. Researchers hope that a new arsenal of techniques — incorporating biomaterials, culturing strategies, surgical devices and more — will translate into delivery methods that help cells survive and integrate appropriately into the human body, as well as limiting unpredictable and potentially dangerous cellular behaviour.



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### The spinal cord

Geron's cells are eventually intended to treat human spinal cord injury. In preclinical research many cell types have been transplanted into the rat spinal cord, the animal of choice. But whether the cells were neural stem cells, haematopoietic stem cells, marrow stromal cells, or cells derived from ESCs and induced pluripotent stem (iPS) cells, all showed the same result: rapid and major cell loss shortly after delivery. "It's a huge challenge," says Molly Shoichet, a bioengineer at the University of Toronto in Canada.

In a recent analysis of three methods of delivering cells to the spinal cord — lumbar puncture, intravenous injection and direct injection into the cord — Birgit Neuhuber and colleagues at Drexel University College of Medicine in Philadelphia, Pennsylvania, found that three weeks after delivery, engraftment of marrow stromal cells into the spinal cord was relatively inefficient by any of the methods<sup>1</sup>. The most invasive method, direct injection, gave the best result (6.1% of delivered cells engrafted after three weeks), followed by lumbar puncture (3.4%) and intravenous injection (1.6%). Similar losses occur when cells are injected into the peripheral nervous system, says Rajiv Midha, a neuroscientist at the University of Calgary in Canada: "Our data suggest that about 95% of cells do not survive." One Canadian study demonstrated improved cell survival, up to 40%, by the addition of a cocktail of growth factors, anti-inflammatory drugs and immunosuppressants<sup>2</sup>.

In the search for better methods, researchers have begun to move beyond simple injection. One approach is to mix the cells with a durable inert matrix. Materials such as hydrogels — natural or synthetic water-insoluble polymers — could provide a scaffold around the spinal cord on which cells might stay put and grow. In one study, Shoichet and colleagues found that injection of cells in a hyaluronan and methylcellulose hydrogel doubled cell survival from about 0.5% to 1%, but the percentages were still so small that they did not publish the work.

In a further experiment, Shoichet and her team created a soft porous hydrogel tube, like a bendy straw full of holes, and seeded it with neural stem/progenitor cells<sup>3</sup>. This arrangement promoted cell survival and differentiation *in vivo*, but not functional recovery, says Shoichet. She is now working to incorporate drugs and growth factors into the tube wall. But attaching tubes to the spinal cord is a highly invasive surgery, and it remains to be seen if even positive functional results from rodents would be worth the risk in humans. Direct injection alone poses major surgical risks. "If you inject into [the spinal cord] are you going to disturb it such that you sever some of the axons?" asks Aileen Anderson, a professor of physical medicine and rehabilitation at the University of California, Irvine. "Are you going to cause local demyelination or inflammation? We don't want to risk taking away any preserved function in the course of trying to do something reparative."

In addition to cell loss, there are other delivery challenges to confront, such as how many cells to inject, where to inject them and the ideal time after an injury to do so. Cells introduced too soon after injury could be slaughtered by the body's inflammatory response. Cells introduced too late could be blocked by scar tissue. Indeed, Geron hopes it has gauged the sweet spot: it is recruiting only newly injured patients into its trial and plans to inject cells 7 to 14 days after injury.

The Geron trial marks not only a beginning for hESC-derived cell trials in the United States, but the first North American trial injecting any sort of stem cells into the human spinal cord. The company declined to be interviewed for this article. According to their website, Geron has developed a "syringe positioning device" for the trial that attaches to an operating table as a way to precisely control cell injection. All participating surgeons will be trained to use it, the website notes.

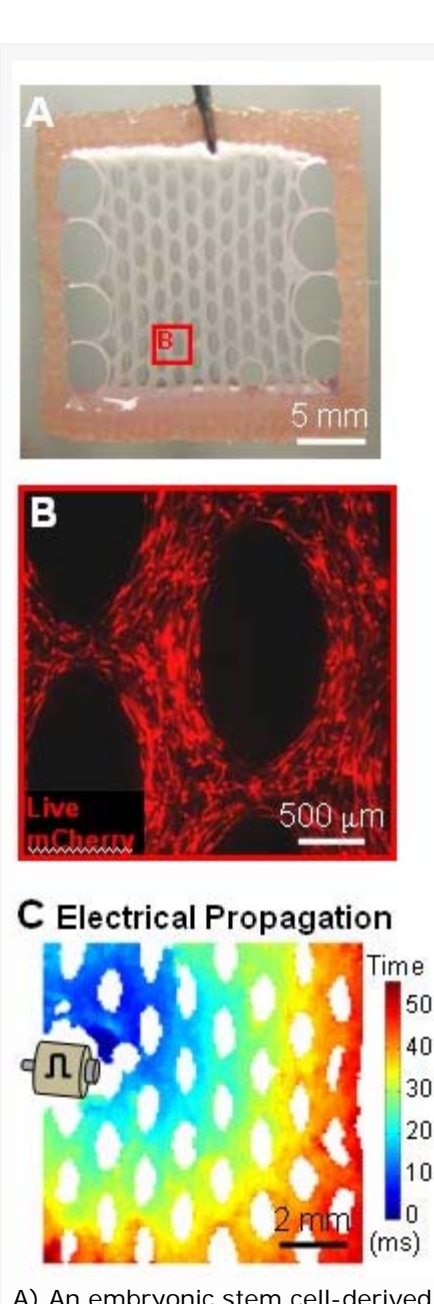
For now, the rest of the community awaits more positive lab data. "In most of the animal experiments, we get very modest amounts of recovery," says Neuhuber. "We're all still trying to see the huge improvement." And until that happens, researchers are unlikely to flood the FDA with clinical trial applications. "Nobody wants to go into the clinic and fail, and then have the entire field blame them for the failure," she says. Several human studies have been done, mainly using cells derived from the patients' own blood or bone marrow, in Russia, Brazil and Japan, but most are not up to FDA standards, and some have been criticized for lack of follow-through. "There aren't controls and the protocols aren't well defined, so it's hard to understand what's going on," says Shoichet. Neuhuber agrees: "Sometimes it's not even clear what kind of cells they're injecting. It's terrible."

And even when a well-documented phase I trial does get off the ground, some expect the real hurdles for spinal cord cell therapies to come with phase II trials, which test efficacy. Spinal cord injuries have highly variable degrees of spontaneous recovery for the first three months after injury, so it is estimated that any phase II trial would require several hundred participants to prove a treatment's efficacy, and there are only about 7,800 new spinal cord injuries each year in the United States, according to the National Spinal Cord Injury Association. "That's going to become a hugely limiting factor very quickly," says Anderson. "That's the next stage of the game."

### The heart

In contrast to the spinal cord, clinical trials for stem cell therapies in the heart are under way in North America and Europe, investigating the potential benefits of skeletal myoblasts, bone marrow cells (BMCs), mesenchymal stem cells (MSCs), and cardiac stem cells derived from heart biopsies.

The heart is one of the least regenerative organs in the body. Heart transplantation is currently the only effective therapy for major cardiac cell loss, and this makes stem cell therapies alluring potential treatments. In early trials in which BMCs and myoblasts were injected into the heart, researchers hoped that these cells, which do not normally become cardiomyocytes, might transdifferentiate to heart muscle. They didn't. However, researchers did document improved cardiac function during the trials, a benefit eventually attributed to transient secretions from the transplanted cells. (See [Stem cells for the heart, a new wave of clinical trials](#).) "Mesenchymal stem cells produce a wide variety of growth factors," says Techung Lee, a biochemist at the State University of New York at Buffalo. "When you inject the stem cells into the host, you are essentially carrying out growth factor therapy." The paracrine action of the cells, however, is not permanent, and the main goal of cardiac cell therapy remains long-term function.



A) An embryonic stem cell-derived cardiac tissue patch inside a Velcro frame. B) Cardiomyocytes (red) in the patch are aligned along the pore boundaries. C) A stimulus electrode (to the left) evokes a response that spreads throughout the patch (from blue to red) within 50 milliseconds.

Nenad Bursac, Duke University

To actually create new muscle, many researchers are turning to ESCs and iPS cells, the only types that have proved capable of differentiating into cardiomyocytes (endogenous adult stem cells have been shown to generate new cardiomyocytes *in vitro* but only in the presence of other cardiomyocytes).

As with the spinal cord, most cells are lost right at the beginning. Cells are generally injected into the coronary artery or the beating heart muscle, explains Sian Harding, a cardiac researcher at Imperial College London. "It's very difficult to get anything to stay in the heart with all the compressive forces," she says. During a 2002–06 phase II randomized, double-blind trial of myoblast injection held in 21 academic hospitals around Europe, researchers found that direct injection was poorly reproducible, and the study raised the possibility that clusters of cells left in the myocardium increase the chance of heart arrhythmias, says lead author Philippe Menasché at the European Hospital Georges Pompidou in Paris<sup>4</sup>. Researchers learned the hard way that "the conventional injection method was probably not ideal," he says.

Researchers are designing alternative methods — hydrogels, bioengineered patches and cell sheets — that might hold the cells in place. One recent pilot study delivered a suspension of human bone marrow-derived MSCs and fibrin glue into the heart of a rat model of myocardial infarction. The combination improved cell retention and survival compared to the same cells delivered in a saline solution<sup>5</sup>.

At Duke University in Durham, North Carolina, Nenad Bursac and colleagues have been working for more than four years to engineer a heart patch seeded with mouse ESC-derived cardiomyocytes that is biocompatible and functional. It's been no easy task. They recently designed a malleable hydrogel sheet pierced with holes, like a block of jello skewered with a hairbrush. Cells put into the gel align along the elliptically shaped pores, says Bursac, an arrangement which not only gives them structural support but also better access to oxygen. And the technique is highly reproducible, he adds, as each hydrogel scaffold is computer designed, similar to the way circuits are printed on circuit boards. The team is now carrying out an extensive functional test in rats. "Without functional integration, we may cause more damage than benefit," says Bursac.

To use an engineered patch in clinical trials, researchers need first to receive approval for use of a foreign material. A faster route might be to use a completely biological construct, with no foreign scaffold. After the marginal results from the myoblast injection trial, Menasché switched to a cell sheet technology developed by Teruo Okano and colleagues at the Tokyo Women's Medical University in Japan. Cells plated on temperature-responsive culture dishes excrete an extracellular matrix and slowly form into a cohesive sheet of cells, which can be carefully stacked with two or three other sheets for increased thickness, and then overlaid on the injured area of the heart<sup>6</sup>. "You can pre-shape the cell sheet as you want," says Menasché, "and [the sheet] probably increases the likelihood that more cells are going remain alive, because they don't lose survival signals."

Poor cell survival and retention could be major factors in the mediocre performance of stem cell clinical trials for heart disease. Results have been "inconsistent," says Lee: some trials fail to show any benefit whereas others claim moderate improvement. As of now, stem cell clinical trials have documented safety, but shown little to no efficacy, agrees Menasché. "But negative trials can be turned into positive trials if we try to understand why we failed."

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