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REPORT

Astonishing Advances in Tissue Regeneration

By Heather S. Oliff, PhD



Stem cells. Groups of human embryonic stem cells (blue and purple) on their feeder cells (green). Human embryonic stem cells are a potential source of cells to repair damaged tissue in diseases such as Parkinson's and insulin-dependent diabetes.

Scientists in the United States, Germany, and other countries are reporting extraordinary progress in regenerating and engineering living tissues in the laboratory. Until recently, many of these remarkable medical breakthroughs would have been considered the stuff of science fiction rather than science.

While placing an order for manmade organs and tissues may still seem like a futuristic concept, many researchers believe that new techniques such as producing complementary DNA libraries, genetic mapping, animal models of regeneration, and stem cell research will one day turn this concept into a reality.

In this report, we discuss some of the latest advances in tissue regeneration and engineering from research labs around the world.

REGENERATIVE CELL TYPES

Proper wound healing is essential to maintaining good health. In humans and other mammals, most injury leads to wound repair, where the area of injury is mended with a functional but different material, such as scar tissue.

A second, rarer form of repair involves regeneration. Regeneration is distinct from repair because it produces tissue that both looks and functions normally. In contrast to animals such as worms, lizards, and starfish that are able to regenerate body parts, humans cannot yet regenerate organs, though they can regenerate some tissues such as bone. Inducing cell and tissue regeneration may well hold the key to successfully treating diseases and reversing aging in humans.

Cells that do not proliferate, or multiply, cannot regenerate. Stem cells are immature tissue precursor cells that can self-renew and differentiate into specific cell types, depending on the signals they receive from their environment.¹ Scientists are now studying two main types of stem cells: embryonic and adult.

UNLIMITED POTENTIAL OF EMBRYONIC STEM CELLS

The use of embryonic stem cells is highly controversial, since obtaining the stem cells requires destruction of the embryo. Embryonic stem cells come from five- to eight-day-old embryos. At this stage, the embryo is a ball of cells smaller than a pinpoint, comprising an outer coat of cells and an inner cell mass. Scientists take the embryonic stem cells from the inner cell mass and grow them in a dish where they multiply, generating the starting material for research studies. Since each embryonic stem cell has the potential to become any cell in the body, the potential applications and benefits of stem cell therapy are virtually unlimited.

Overcoming the ethical dilemmas surrounding embryonic stem cell research is paramount to advancement of the field. A dramatic breakthrough was noted last fall in the prestigious scientific journal *Nature*. Scientists at Advanced Cell Technology in Worcester, MA, reported that they had developed, for the first time, a way to remove a single viable embryonic stem cell from an embryo without damaging the embryo's ability to develop into a full-term organism.²

This technique is similar to that commonly used in in-vitro fertilization, where genetic defects are determined by extracting a single cell before the embryo is implanted in the woman.² One day it might be possible for people to bank their own embryonic stem cells so that a damaged organ could be replaced with a new organ grown with their own cells.

ADULT STEM CELLS ALSO HOLD VALUE

At one time, scientists believed that adult stem cells were present only in bone marrow, the soft material that fills the inside cavity of bones. Now investigators have established that adult stem cells are present in many tissues and organs, and that they can differentiate into numerous types of cells.

Unlike embryonic stem cells, adult stem cells cannot grow into any type of cell; instead, their differentiation is guided by their embryonic origins and the type of tissue in which they reside.¹ For example, adult stem cells in the small intestine help create epithelial cells that line the lumen (inside) of the intestine.¹ Adult stem cells also reside in tissues found in the spinal cord, brain, blood, bone marrow, dental pulp, blood vessels, skeletal muscle, epithelial layer of the skin, cornea, retina, liver, and pancreas.¹



Because many of these tissues are adversely affected by the process of aging, adult stem cells also hold vast potential to improve health and quality of life with age. Recently, scientists discovered that adult stem cells exhibit plasticity, meaning that cells from one tissue can generate functional cell types of another tissue. This phenomenon may eventually be used to generate new tissues or organs for transplantation.¹

POP SINGER UNDERGOES SUCCESSFUL STEM CELL OPERATION

Hawaiian singer Don Ho underwent an experimental stem cell procedure for his heart in a Thailand hospital in December 2005. Best known for the pop hit “Tiny Bubbles,” the 75-year-old singer had suffered from heart problems, including shortness of breath and arrhythmias, for about a year prior to undergoing the procedure. Ho received a pacemaker implant several months before the stem cell procedure.⁸

The experimental procedure, which is not yet approved in the United States, involves multiplying stem cells taken from the blood and injecting them into the heart with the goal of strengthening the organ. Similar stem cell treatments have been used in Europe for about two years, and FDA-approved studies are under way in the US.

Following the procedure, Ho reported that he was feeling much better and hoped to return to the stage soon.⁸

REGENERATING A DAMAGED HEART

It is well known that cardiovascular disease, which comprises heart or circulatory system impairments, is a major cause of death and disability. When heart muscle cells known as cardiomyocytes die, the heart weakens and cannot pump as efficiently. Cardiomyocytes may be destroyed by high blood pressure, atherosclerosis, or heart attack.¹ In addition to functioning cardiomyocytes, a healthy cardiovascular system requires vascular endothelial cells and smooth muscle cells to form the blood vessels that supply the heart muscle with blood, oxygen, and energy. For many people, repairing the cardiovascular system is a major key to longevity.

Fortunately, stem cells can differentiate into new cardiomyocytes and vascular endothelial cells. Researchers at prestigious institutions such as Johns Hopkins, New York Medical College, and the National Institutes of Health first tested the ability of stem cells to restore heart function in animals. In mice and pigs with experimentally induced heart attacks, stem cells from bone marrow were injected into the heart or blood circulation.³⁻⁶ Stem cells injected into the circulation migrated to the damaged area of the heart, preferentially attracted to the damaged rather than the healthy tissue.⁴ The stem cells stimulated the formation of new cardiomyocytes, vascular endothelium cells, and smooth muscle cells, helping to replace the damaged heart and vessel tissue with new tissue.⁵ One study reported that 68% of the damaged heart was replaced with newly formed heart muscle,⁶ while others noted that the damaged hearts could contract better after the stem cell infusion.^{3,5} Johns Hopkins researchers also demonstrated that human adult bone marrow stem cells injected into a mouse can enter the heart muscle and differentiate into cardiomyocytes.⁷ This confirms that human adult stem cells can differentiate into new heart cells in vivo. Clinical studies of these technologies soon followed.

A small study at the University of Frankfurt in Germany compared the effect of bone marrow-derived and blood-derived progenitor cells, which are similar to stem cells, in people approximately four days after a heart attack.⁹ Cells were placed in the heart with a balloon catheter. A “balloon” is used to block blood flow temporarily so the cells can have maximum contact time with the damaged heart. At the four-month follow-up, the patients demonstrated improved left ventricular heart function, complete normalization of coronary blood flow reserve, and improved contractibility of the infarcted heart muscle. Both cell sources were equally effective. A group of patients who did not receive cell therapy did not report similar improvements.⁹ Long-term follow-up is needed to determine whether these benefits persist beyond four months.

Two other small clinical studies conducted in Germany used the balloon catheter method to inject autologous (donor and recipient are the same person) bone marrow stem cells directly into the damaged infarct zone in patients who had suffered a

heart attack five to nine days earlier. For purposes of comparison, some patients were treated with standard therapy instead of stem cell therapy. After three months, the stem cell therapy group demonstrated a significant decrease in the size of the infarct region, as well as an increase in left ventricular function.^{10,11} The researchers concluded that autologous stem cell therapy is safe and effective in supporting the regeneration of myocardial tissue and function.¹¹ Another similar study reported positive findings, though the researchers used a different method of delivering autologous bone marrow stem cells with a catheter (small tube) that was placed in the heart through a puncture in the femoral (thigh) artery.¹²

Randomized, controlled clinical trials are considered the gold standard in assessing the efficacy of innovative technologies. Researchers conducted the first randomized, controlled clinical trial of stem cell therapy at Hanover Medical School in Hanover, Germany, in 2004. Within five days of suffering a heart attack, patients were randomly assigned to receive standard medical therapy either alone or in combination with autologous bone marrow stem cells injected into the infarct-related artery. Left ventricular function improved by 7% in the cell therapy group and by less than 1% in the control group. Intracoronary transfer of bone marrow stem cells thus appears to improve heart function following heart attack.¹³ Further investigations are indicated to determine whether stem cell therapy may also improve survival rates following myocardial infarction.



Dorsal view of the nerves of the central and peripheral nervous systems.

TREATING CENTRAL NERVOUS SYSTEM DEFICITS

A noted television commercial from the 1980s portrays the brain as an egg, and an egg sizzling in a frying pan as the “brain on drugs.” The commercial’s purpose was to warn viewers that a brain “cooked” by drugs could be damaged beyond repair. This campaign reflected the medical knowledge of the time, which purported that cells of the brain and spinal cord called neurons were not able to regenerate, and thus that dead neurons were gone for good.

Today, however, scientists understand that some parts of the brain can regenerate, and that new neurons can develop from neural stem cells.¹ Neural stem cells can also give rise to critical nervous system support cells called oligodendrocytes, astrocytes, and glial cells. Scientists believe that in the future, stem cell therapies may be able to cure nervous system conditions such as Parkinson’s disease, brain and spinal cord damage from stroke or trauma, Huntington’s disease, multiple sclerosis, epilepsy, and amyotrophic lateral sclerosis (Lou Gehrig’s disease).

Scientists are now investigating three main avenues of central nervous system regeneration: 1) growing neural stem cells in a dish and implanting them into the body after they have differentiated into the needed cells; 2) implanting neural stem cells directly into the body where they can differentiate into the needed cells; and 3) finding growth factors and hormones that can signal the patient’s own neural stem cells to differentiate.¹ Since stem cells require environmental cues to guide what type of cells they will become, researchers are investigating how to manipulate the signals that determine stem cell behavior. The ability to use neural stem cells may depend on scientists’ ability to modify such signals.

In a recent study at the University of California, Irvine, researchers induced human embryonic stem cells to differentiate into oligodendrocyte progenitor cells in the laboratory. Oligodendrocytes help form the myelin sheath that insulates neurons. Loss of this myelin sheath can contribute to impaired movement following spinal cord injury. When the cells were transplanted into rats with spinal cord injuries, the animals demonstrated a restoration of myelin damaged by the injury, and regained their ability to walk.¹⁴ This promising finding offers hope that stem cell therapies may one day help people who suffer from spinal cord injuries to regain lost motor skills, such as the ability to walk.

OF MICE AND MEN: HUMAN STEM CELLS TRANSPLANTED INTO MICE BRAINS

In a groundbreaking development in the field of stem cell research, investigators at the Salk Institute for Biological Sciences in La Jolla, CA, showed that human embryonic stem cells implanted into the brain ventricles of embryonic mice can differentiate into mature, active human neurons that successfully integrate into the adult mouse brain.

While human embryonic stem cells can theoretically develop into any kind of cell, differentiation of these cells had previously been demonstrated only in culture or in teratoma (tumor) formation. The California researchers also revealed that signals that stimulate neural differentiation are similar between different mammals.¹⁵

The researchers injected 100,000 human embryonic stem cells into the brains of 14-day-old mouse embryos. The mice were born with about 0.1% of human cells in their brains. This study represents a major step in overcoming the technical challenge of when to inject stem cells into recipients. The results suggest that human embryonic stem cells will mature similarly to the cells that surround them.¹⁵

This new model, combining characteristics of mice and men, will allow human neural development to be studied in a live environment, and may lead to new models for investigating human neurodegenerative and psychiatric diseases. Additionally,

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A GENETIC APPROACH TO REGENERATION

Researchers at the Wistar Institute in Philadelphia, PA, are studying a unique strain of mouse that can heal wounds by regeneration. After a hole is pierced in the mouse's ear (a typical laboratory identification procedure), it closes with no evidence that a hole was ever present.¹⁶ These animals, known as Murphy/Roths/Large mice, or MRL mice, are so named to denote the two scientists who originally bred them, as well as their unusually large size. MRL mice are genetically unique, and scientists are researching them to elucidate the genetics of regeneration, hoping to gather information that can be used to help humans.¹⁷

When the Wistar scientists induced heart injury in both MRL mice and typical mice, they found that the MRL mouse heart returned to normal, whereas the typical mouse heart was scarred.¹⁸ Human hearts scar following injury from heart attack, and the scarring response contributes to chronic heart disease and death.¹⁹ The healing response in the MRL mouse, however, differed greatly from that of the typical mouse. The MRL mouse displayed early movement of cardiomyocytes into the wound site, and DNA synthesis and proliferation of these cells.¹⁸ The MRL mouse heart also demonstrated better revascularization (restoration of blood supply) at the site of injury, which is necessary to help cells thrive and avoid death. According to the scientists, the MRL mouse studies demonstrate that "mammalian hearts have significant capacity to regenerate."¹⁸

The Wistar scientists are now working to identify which genetic and biochemical factors are involved in this regenerative response. They have already identified areas on several chromosomes that control wound closure and are involved in regeneration of the MRL mouse ear tissue.^{19,20} It is unclear whether these same chromosomes are responsible for regenerating the MRL heart.¹⁸

A potential key mediator of regeneration is the family of enzymes known as the matrix metalloproteinases. These protein-digesting enzymes degrade the collagen that helps form scar tissue. They occur in immune cells, along with another family of molecules called the tissue inhibitors of metalloproteinase, which inhibit matrix metalloproteinases. After an injury, neutrophils that contain matrix metalloproteinases and tissue inhibitors of metalloproteinase enter the wound. Regeneration or scarring occurs depending on whether matrix metalloproteinases or tissue inhibitors of metalloproteinase dominate. The MRL mouse ear wound has a more active form of matrix metalloproteinases and lower levels of tissue inhibitors of metalloproteinase than the typical mouse ear wound.¹⁹ This combination promotes a regeneration process rather than a scarring process in the MRL mouse.¹⁹



The scientists also looked at the ability of MRL mice to heal central nervous system injuries.²² In the MRL mice, the matrix metalloproteinase response was temporarily increased following a brain injury, but the brain was not repaired differently than that of the typical mouse.²² The researchers hypothesize that the central nervous system has mechanisms to decrease the matrix metalloproteinase response, and that the tendency to scar blocks regenerative healing.^{17,19,22} Discovering how to prevent the formation of scar tissue may eventually make it possible to regenerate the heart, heal chronic wounds and burns, repair spinal tissue, and promote organ replacement.

NIH GRANT TO ESTABLISH NATIONAL STEM CELL BANK

The National Institutes of Health (NIH) has awarded \$16.1 million over four years to fund a National Stem Cell Bank, NIH director Elias A. Zerhouni, MD, recently announced.

The National Stem Cell Bank, awarded to the WiCell Research Institute in Madison, WI, will be the nation's first and only stem cell bank. As such, WiCell will be the keeper of all federally approved human embryonic stem cell lines, conducting molecular characterizations on each cell line, defining their growth properties, performing quality control, and distributing the cell lines to qualified research scientists worldwide.²³

The WiCell Research Institute is a nonprofit organization founded in 1999 by James Thompson, PhD, a reproductive biologist who was the first to isolate human embryonic stem cell lines. Human embryonic stem cells are derived from embryos approximately six days after their fertilization in the laboratory, as part of an assisted reproductive program for infertile couples. Embryos potentially serving as a source of human embryonic stem cell lines are in excess of those required by the couples

from which they were derived and are therefore destined to be discarded. There are currently more than 400,000 such surplus embryos in the US.

Because human embryonic stem cell lines are capable of self-renewal and propagating daughter cells with the potential to give rise to all tissue types, they have enormous potential in treating many currently untreatable diseases and medical conditions, such as type I diabetes mellitus, Parkinson's disease, and spinal cord injuries. Current guidelines, established jointly by the National Research Council and the Institute of Medicine, provide for human embryonic stem cell line research in treating disease, while prohibiting human cloning.²³

In addition to the WiCell grant, the NIH appointed the University of California, Davis, and Northwestern University as Centers of Excellence in Translational Human Stem Cell Research. The two Centers of Excellence will bring together stem cell experts, disease specialists, and other scientists to explore the use of human stem cells in treating a wide range of disease conditions.²³

TISSUE ENGINEERING HOLDS PROMISE

Millions of dollars are spent each year to develop tissue engineering products and procedures. In fact, some engineered tissues have already been approved by the FDA. One of the first tissues to be engineered and used clinically is bone. Engineered bones, cartilage, tendons, and ligaments may benefit people who suffer from bones that will not fuse, defective tendons, or arthritic joints, as well as those who need dental implants (which require strong bone tissue). These regenerated tissues will one day eliminate the need for standard therapy, which includes stainless steel, cobalt chrome, and bone grafting.

Scientists are also developing engineered skin, which will help treat massive burns, chronic problem wounds that are difficult to heal (common in people with diabetes), and vitiligo (a disease of discolored skin). Although heart valves have been engineered, the valves failed when they were implanted.²⁴ A whole bladder has been engineered and transplanted in a dog.²⁵ The bladder appeared to be normal and demonstrated normal function.²⁵ An engineered bladder has not been evaluated in humans. Nearly every body tissue is being engineered for future applications in medicine.

Three components are needed for successful tissue engineering: cells (such as stem cells), scaffold or matrix (which provides a degradable physical base for cell growth), and growth factors.²⁶ Simply put, the cells grow along a physical scaffold, and specific growth factors stimulate cell activity and differentiation into the desired tissue.²⁶

Three main techniques are now being studied : 1) injecting cells into the damaged tissue, either with or without a degradable scaffold; 2) growing a complete three-dimensional tissue to maturity in the laboratory and then implanting it into the patient; and 3) implanting a scaffold directly into the injured tissue, stimulating the body's own cells to regenerate the tissue.²⁷



Many challenges to achieving successful tissue engineering remain, however. For example, once it is placed into the body, the engineered tissue must be supplied with blood. New blood vessels must form quickly or the tissue will die. This presents a greater challenge in larger engineered tissues. The timing and appropriate doses of growth factors are still under investigation. Scientists are also developing optimal scaffolds that can guide the growth of cells within the patient.²⁷

CONCLUSION

Remarkable advances in tissue regeneration and engineering hold great promise for curing diseases and prolonging life. One day, scientists and physicians may use stem cell therapies to regenerate damaged tissues and organs or to cure conditions such as Parkinson's disease, arthritis, and diabetes. They may also be used to reverse the aging process.

As research into these extraordinary technologies continues to accelerate, the day when these possibilities become realities draws ever closer.

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