Regenerative Medicine

Healing Promise

Luke Massella was born with spina bifida, a birth defect involving the spinal column. In 2001 he was seriously ill. He couldn’t run around or play outside with his friends. He could barely go to school. In Luke’s case, the defect in his spinal column led to a paralyzed bladder. When the bladder does not function properly, urine can back up and cause damage to the kidneys, which are responsible for filtering waste from the blood. When the kidneys don’t work, toxins build up. Kidney damage often is life-threatening. Even after 16 surgeries, Luke’s kidneys were in danger. He was losing weight and still was unable to live a normal life. Then, Luke received a new bladder grown outside his body using techniques from the new and experimental field of regenerative medicine. This preserved his kidneys and restored his health. He even became captain of his high school wrestling team. In 2012 Luke is enjoying life as a healthy, athletic college student. Luke’s doctor for this groundbreaking treatment was Dr. Anthony Atala, Director of the Wake Forest Institute for Regenerative Medicine (WFIRM), in Winston-Salem, N.C. (See the Resources section later in this chapter for links to TED Talk videos of Dr. Atala explaining his techniques and meeting with Luke 10 years after Luke received a new bladder.)

Regenerative medicine is still in its infancy. Luke’s miraculous story was part of a clinical trial; his treatment won’t become routine for several more years. However, research being done today in North Carolina and around the world is solving problems that will lead to new treatments for patients with many different damaged and diseased organs. Damaged and diseased organs are a huge medical challenge. Sometimes doctors can repair or replace damaged organs with artificial parts, but these artificial parts (such as a titanium hip or artificial heart valve) can deteriorate over time and may cause infection or inflammation. Artificial replacement parts are a particular problem for children because they don’t grow with the child, so these replacement parts have to be replaced again as the child grows. Human organ transplants from living or dead donors are another way to treat severely damaged organs, but organ transplants pose many issues and are definitely not a cure-all.

In 2011, there were 28,537 organ transplants in the United States, 1,042 of which were done in North Carolina. Sadly, this does not begin to meet the need for such transplants. More than 100,000 people in the U.S. are waiting for an organ transplant — the majority for a kidney. An average of 18 people die each
day while waiting. Furthermore, even a successful transplant leads to a lifetime of special drugs to keep the body from rejecting the donated organ. The fantastic promise of regenerative medicine is that someday doctors will be able to heal patients with cell and gene therapies and grow replacement tissues and organs from a patient’s own cells. If doctors are able to grow replacement tissues from a patient’s own cells, the patient’s immune system will accept the transplant without powerful immune suppressant drugs, and the patient will be able to live a much more normal life. Let’s take a look at some of the science behind this promise.

**Background Science**

**Tissues and Organs**

Tissues are groups of similar cells performing a similar function. Organs are made up of multiple tissues working together to perform a function. Scientists are researching ways to regenerate more than 30 different tissues and organs, including skin, blood vessels, bladders, bone, kidneys, lungs and livers. Organs range in form and complexity from flat organs (such as skin) to tubes (such as blood vessels or the ureters) to hollow, bag-like organs to complex, solid organs. For example, the bladder is a hollow, bag-shaped organ lined with smooth epithelial tissue on the inside and smooth muscle tissue on the outside. Solid organs such as the kidney and liver are more complex. The kidney has multiple specialized parts to filter waste from the blood and excrete it as urine. So far, regenerative medicine techniques successfully have been used to replace damaged skin, cartilage, the urethra, the bladder and the trachea in human patients. However, all these treatments remain experimental. Many questions about materials, safety and procedure still must be answered before regenerative treatments that replace these organs become standard medical practice. It will be even longer before a complex, solid organ such as the kidney can be regenerated successfully and placed in a human patient.
The Extracellular Matrix: A Scaffold for Building New Organs

The first step of building replacement organs is to build a scaffold. Simple tissues and complicated organs are similar in that they create and reside in a supportive extracellular matrix. This extracellular matrix is outside the cells and consists of proteins and polysaccharides. The polysaccharides are linked to proteins to form a gel-like substance in which other fibrous proteins are embedded. The gel allows diffusion of nutrients, wastes and other chemicals to and from the cells. The fibrous proteins form a strong, resilient scaffold and help organize the cells. Amazingly, tissues and organs can be decellularized. In other words, all the cells can be removed, leaving only the extracellular matrix. The matrix forms a scaffold for the cells but is not itself made of living tissue. This matrix then can be used as a scaffold to build a new organ.

A scanning electron micrograph of native extracellular matrix in connective tissue. It is largely composed of collagen fibrils. The hydrogel, composed of proteoglycans and glycosaminoglycans, that normally fills the interstices of this fibrous network has been removed by the processing treatment.

To build a new tissue or organ, researchers place new cells of the desired types in the correct location on the scaffold. Then they grow the cells on the scaffold in a growth medium for several weeks. The scaffold is important not only because it provides support, but also because it influences where and how the cells grow. This helps orient the cells correctly for their function. Scaffolds can come from deceased human donors or animal organs, or they can be built from synthetic biomaterial. All of these are much more available than human organs suitable for transplant.
Challenges
Researchers studying these scaffolds are working on several challenges. One important challenge is developing biomaterials to build artificial scaffolds. These biomaterials must be nonreactive with the human immune system. They need to have the right texture to signal cells to grow and orient themselves correctly. And they need to be strong enough to last until the new organ creates its own extracellular matrix, then dissolve away like surgical sutures. Researchers also are investigating the effects of embedding various growth factors or anti-inflammatory medications in the scaffolds.

Once the scaffold materials are developed, the next challenge is building the scaffold. Just as 3D printers can make solid objects by laying down layers of plastic, 3D bioprinters are being developed that one day may be able to build human replacement organs. In ink printers, different colors of ink are kept in separate cartridges and printed together to form the exact desired color. Similarly, bioprinters can keep cells and different substances separate until placing them exactly where needed in the new tissue. Dr. Atala and the research team at the WFIRM recently demonstrated how this might work by printing out a model of a kidney with cells. However, much more research is needed before this experimental technique will be ready to build a functional kidney that can be used safely in patients.

The decellularization of animal tissues presents a different challenge: removing all the cells without damaging the function of the scaffold. This is difficult because the scaffold not only needs to have the right structure, but it also must have the right texture and the right chemical properties. Different tissues require different techniques, and these different techniques may affect the structure and composition of the scaffold in different ways. Researchers are experimenting with a variety of detergents and enzymes as well as with different protocols to perfuse the tissue and remove the cells.

Growing new cells on the scaffold and preparing the tissue or organ for its role within the body also is challenging. Growing human cells outside the body was a huge problem. Researchers are finding that many tissues have some undifferentiated cells that will reproduce and grow in the right environment.
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and with the right nutrients in the culture media. Experimentation with growth factors is leading to improved control of cell proliferation and differentiation. Researchers also are designing equipment to simulate the normal environment of the body with hydrostatic pressure, pulsing fluid flow and stretching and compressing tissues. This exercises and conditions the tissues to their environment and helps signal the growing cells to organize themselves correctly. For example, researchers grow a regenerated heart valve in a tube and pump the growth medium through the tube to simulate the rate and pressure of blood flow. Such laboratory devices are referred to as bioreactors. They play a key role in the regenerative medicine process.

**Stem Cells**
A variety of regenerative therapies, including production of cells to populate the extracellular matrix, depend on stem cells. Stem cells have been extremely controversial in the political arena, yet many people do not understand what they really are or why they may lead to exciting advances in medicine.

Think about your body. It is composed of many different types of cells. You already may be familiar with neurons, red blood cells and muscle cells. All these different cells must be generated from the zygote, a single cell formed by the joining of a single egg and sperm. As multicellular organisms develop from zygotes to adults, they must produce differentiated cells capable of forming all the organism's different tissues and organs. The undifferentiated cells that give rise to other types of cells are called **stem cells**. There are many different types of stem cells found at different stages of development and in different parts of the body. The hope is that these cells can be used to repair tissues and grow new organs — but to do this we must understand how these cells work. Researchers are beginning to learn how development and differentiation are controlled at the molecular level.

When a zygote first begins to grow, it is **totipotent**. This one cell can give rise to all the tissues needed for the body as well as the cell types needed for the extra embryonic tissues, such as the placenta. As the zygote divides and goes through the various stages of development, the cells begin to differentiate. The differentiation is controlled by chemical signals that cause changes in cell epigenetics. In an epigenetic change, the sequence of the nucleotides (ACGT)
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is unchanged, but chemical changes in the chromosome turn on or off particular genes. *(See the Resources section later in this chapter for a link to more about epigenetics.)* These genes then stay on or off even as the cell divides so that the changes are passed on to the daughter cells. This means that normally once a cell has differentiated into one type of cell (a nerve cell, for example) it can’t differentiate backward into another type of cell. Therefore, even though each cell in an organism has all the information for all the types of cells found in that organism, only some of this information is available to the cell.

Embryonic stem cells are **pluripotent**. Pluripotent cells can give rise to all the other types of body cells. Human embryonic stem (hES) cells are derived from human embryos created as a part of the *in vitro* fertilization process at fertility clinics. The embryonic stem cell lines come from extra embryos donated for research purposes. The hES cells come from cells taken from the blastocyst stage of the embryos. Because this has the potential to save lives but also destroys these embryos, creation of new embryonic stem cells has been the subject of much ethical and legal debate.

Adult stem cells (also called somatic stem cells) are undifferentiated cells found in differentiated tissues of children and adults. These stem cells are **multipotent**. They can give rise to the multiple cell types needed in the tissue
they come from, but due to epigenetic control they are no longer pluripotent. Until the mid-2000s, most types of adult stem cells were difficult to find and work with, and little was known about them. Only stem cells found in the bone marrow (hematopoietic stem cells and bone marrow stromal cells) currently are used in standard medical treatments.

Hematopoietic (blood) stem cells have been used successfully for years to treat various blood disorders such as leukemia and lymphoma. In these cases, stem cells from the patient or donor’s bone marrow replace diseased bone marrow cells. Treatments using other types of stem cells are mostly in preclinical stages or early clinical trials. Even the mechanisms by which the stem cells might help are not yet well understood. Stem cells may help replace diseased tissue either by integrating with the tissue and producing new cells or by producing growth factors that cause the patient’s cells to regenerate and repair themselves.

Researchers also are experimenting with various approaches to regrowing skin on burn patients. First, skin stem cells are isolated from an unburned area on the patient’s own skin. Then, the printer or spray gun is used to place the skin stem cells and other skin cells directly on the burn. A special bandage that provides nutrient fluids and clears wastes supports the healing tissue. The stem cells provide growth factors to the damaged skin so it can regrow rather than integrating with it and becoming part of the new skin. The growth factors promote healing, and although the spray gun has not yet been used in humans, initial results show these types of techniques can promote much faster recovery from serious burns.

Many other researchers also are working to develop new treatments using stem cells. In 2012, there exist clinical trials testing the safety of using retinal cells derived from human embryonic stem cells to treat two progressive eye diseases that usually result in blindness. Other clinical trials are testing the use of stem cells to treat heart disease, diabetes and many other diseases.

In 2006, Japanese researchers published the first report of induced pluripotent stem cells (iPSCs) in mice. In 2007, that group and two others published results indicating they had created pluripotent stem cells from adult cells in humans.
They did this by using viruses to insert genes for transcription factors into the DNA of various types of cells. This seemed to reprogram the cells back to an undifferentiated state. The resulting cells then could be cultured and induced to differentiate into adult cells of various types—e.g., beating heart muscle. These induced pluripotent stem cells are exciting because researchers are able to use them to create cultures of tissues from organisms with various diseases, which allows for *in vitro* studies of disease processes and potential drug treatments. They also increase the potential for growing replacement tissues or even organs from a patient’s own cells, which reduces the likelihood of rejection. iPSCs already have been successful in treating blood disorders in mice. Unfortunately, the reprogrammed cells are not exactly like embryonic stem cells. They do not always behave in the same way, they have different epigenetic markers and they sometimes lead to tumors in experimental animals. More research is needed to understand how to control the programming of these cells.

Another newly discovered source of stem cells is human amniotic fluid. Amniotic fluid is the fluid that surrounds the developing baby in the womb. Amniotic fluid-derived stem cells come from the amniotic fluid taken in an amniocentesis or naturally produced at birth. Thus, they do not involve destroying an embryo. They are multipotent and can form all sorts of tissues. Unlike embryonic stem cells, they do not form tumors when grown in animals. Scientists are continuing to study the effects of various growth factors on the growth and differentiation of amniotic fluid stem cells when placed into various types of tissues and scaffolds.

**Engineering New Bone Tissue**

Dr. Elizabeth Lobo and her research team at North Carolina State University’s Cell Mechanics Laboratory are doing work that will lead to better understanding of bone and muscle regeneration. This team is studying the effects of the mechanical environment on bone formation. In addition to regulation by transcription factors, stem cells found in bone respond to electrical signals,
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the physical environment and mechanical signals — including the amount and
direction of stress, tensile strain (pulling), compression and hydrostatic pressure.
For example, the right amount of tensile strain on these stem cells results in
formation of new bone tissue, while greater strain results in scar tissue. Bone
cells have a variety of receptor molecules that cross the cell membrane and
respond to these changes in the mechanical environment by changing shape.
This presents new binding sites and sets off biochemical changes within the
cell. (This translation of a mechanical signal to a biochemical signal is called
mechanotransduction.) Physical properties of the extracellular membrane,
such as fiber diameter, stiffness and the size of niches in which cells can
settle, also are important. Researchers are investigating scaffold characteristics
and scaffolds that slowly can release medications to speed healing, reduce
inflammation and prevent infection. Eventually, this research will lead to new
treatments for wounded veterans and others who have lost or damaged bones, as
well as for infants born with bone deformities.

Careers in Regenerative Medicine

Regenerative medicine depends on bringing together fundamental research
from many areas of medicine and on moving research from basic science to
animal trials to clinical trials in humans and patient care. This can be done
more efficiently with a large, coordinated team approach than with the more
traditional academic departments in which each senior scientist leads an
independent research team. At the WFIRM, for example, teams of scientists
working together include molecular biologists, cell biologists, physiologists,
pharmacologists, biomedical engineers, surgeons, veterinarians and many more.

Focus on Technicians: Jay Barrett
Jay Barrett is a Core Technician at the WFIRM. He supports a large group of
researchers. His responsibilities include managing operations and equipment,
supplies control, giving tours and keeping records. Because WFIRM researchers
share laboratory space and equipment, it is Jay’s job to maintain systems that
ensure people keep the laboratory clean and organized. He maintains and repairs machines as needed and enjoys experimenting with the electrospinning machine that is used to build scaffolds. Jay’s first degree was in business, but he found his career in finance unfulfilling and went back to school to get an Associate degree in biotechnology from Forsyth Technical Community College. At Forsyth Tech, Jay took courses in cell culture, bioprocessing, statistics and aseptic techniques. As part of that program, he was able to do an internship at the WFRIM, where he became hooked on the excitement of the science of regenerative medicine.

Jay’s advice to students is to do as well as possible in each subject because this will lead to better opportunities. He thinks it is especially important to study statistics because it is so important to the analysis of data in every field of research. He also thinks it is essential to continue learning. Jay enthusiastically attends all the weekly research seminars at the WFRIM. This gives him the opportunity to learn the latest techniques and findings from world-class researchers.

Focus on Scientists: Elizabeth Lobo
Dr. Elizabeth Lobo is the Associate Chair and an Associate Professor in the Joint Department of Biomedical Engineering at the University of North Carolina at Chapel Hill and North Carolina State University. She also is an Associate Professor in Materials Science and Engineering at NCSU and the founding director of the Cell Mechanics Laboratory in the UNC-CH/NCSU Biomedical Engineering department. Dr. Lobo studies how physical stimuli regulate stem cells. Her research group is studying the mechanical signaling pathways involved in bone regeneration. This can be important for bone healing and creating bone implants. They are studying the effects of tension, pressure and electrical and chemical signals, as well as the effects of various scaffold structures on bone cell growth and differentiation.

Dr. Lobo became interested in engineering at Modesto (California) Junior College. She then took a biology course and became fascinated by the idea of applying a mechanical engineering approach to biological and especially