a magazine of biotechnology applications in healthcare, agriculture, the environment, and industry

Tissue Engineering
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On the Cover: In the future of tissue engineering, a computer will help design human body replacement parts using specially grown and engineered cells.
Ancient Greeks told a tale about how the king of the gods, Zeus, punished Prometheus for stealing fire and giving it to people. Zeus tied Prometheus to a cliff and sent an eagle to eat Prometheus’s liver during the day. But every night, the “immortal” liver grew back to its original size.

In truth, if part of our liver is destroyed, it can heal and grow back to the same size. Likewise, our skin heals after a cut and broken bones mend. This ability of our body to repair itself is called regeneration. Throughout the ages, people have wondered why some parts of our bodies regenerate and others, such as nerves and intestines, don’t.

The answer lies in the nature of the tissue. A tissue is a group of specialized cells that do a unique job. Your body has a huge variety of tissues, and each tissue looks completely different from those with other functions in the body. In addition, most tissues work in a larger unit called an organ, such as your brain, liver, stomach, and skin. Each organ has a unique shape and structure specifically designed so it can perform its function in your body. For example, specialized cells called neurons have long extensions that pass along electrical signals. Together, these cells form a tissue that processes the many complicated signals to and from the body. This tissue works alongside blood vessels, membranes, and connective tissues in the organ we call the brain.

Scientists are studying the way your own body builds its many specialized tissues and uses them to construct complex organs. They are learning how the cells surrounding a tissue affect the way that tissue develops, how it functions when healthy, and how it can heal when it is injured. They are using this understanding to make or “engineer” tissues that can function properly in the body. Their success will bring lifesaving relief to people whose tissues or organs are too badly damaged to heal themselves.

This field, called tissue engineering, is still very new. Some of its applications are already making breakthroughs in the way doctors can treat damaged skin, cartilage, and bones. The work on more complicated organ tissue, such as muscles, heart, and liver, is just beginning. In this issue of Your World/Our World, you will read about this exciting field. Put on your hard hats, because we’re heading to a tissue construction site!
Your Body, Your House

Each room in your house has several different “systems”, such as plumbing, electricity, and flooring. Each system has a particular structure that helps it perform its function. For instance, the long, strong, round pipes of the plumbing system are specifically designed for the job of carrying water and sewage.

An organ in your body is like a room in a house, made up of several tissues. For example, your heart has muscles, blood vessels, valves, membranes, nerves, and connective tissues. All these tissues have a unique structure that allows the heart to function. The flexible tubes of blood vessels let blood flow through them, for example, while the muscle fibers stretch and contract to pump the blood.

Support Scaffolding

Carpenters use scaffolding so they can place materials in the right place on a building. A tissue also has a scaffold that supports its ongoing construction. The scaffold determines the three-dimensional shape that the tissue will take, such as whether it will be part of a knobby knuckle, a long shin bone, or a round eyeball. Scientists call this scaffold the extracellular matrix because it is made of material outside the cells—“extracellular”—and a matrix is a 3-D structure with spaces to be filled.

A tissue’s scaffold is made of material uniquely suited to encourage a particular quality in the tissue’s cells. For example, skin has a jelly-like scaffold material with collagen, a protein that gives the tissue an elastic quality. Bone scaffold also contains collagen, but during development the bone cells deposit calcium and proteins in the scaffold, giving the bone rigidity and strength.

Tissue engineers are now learning to imitate a tissue’s natural scaffold. They create a biomaterial, which is a human-made substance that mimics the structure and function of a living (“bio”) material. A biomaterial scaffold is like a honeycomb that provides the outline shape the tissue will grow to fill. But first, tissue engineers grow cells of that tissue type in a culture, which is a nutrient-rich fluid that allows cells to divide many times to create a large number of identical cells.

When cells grow in a culture dish or flask, they act like individual cells. But when they grow in a three-dimensional scaffold, they...
Cells are placed in a biomaterial scaffold, within which they can multiply and form a developing tissue.
A Colony of Tissues

In a way, tissues are like members of an ant colony. Different members have separate functions to keep the colony alive and healthy. Ants start out the same, but they become specialized as they develop. Some become workers, guards, or caretakers of the young, and one becomes the queen. Yet they all work together for the good of the colony.

Likewise, our many tissues develop from the same fertilized human egg. The cells that become brain tissue, lips, and liver all start out the same. Through a series of divisions, they become different types of cells with unique structures and functions. This process is called differentiation.

The cells in liver and skin look different because they have different functions. Yet they both developed from the same undifferentiated cells in the embryo.
Bone marrow stem cells are very valuable for rebuilding the immune system after drug or radiation treatment has destroyed a patient’s bone marrow.

For tissue engineering to succeed, we need to know more about how cells differentiate and develop into individual tissues. Like plants in a garden coming up at certain times throughout spring, summer, and fall, each tissue has an expected time and place of development. Guiding this development requires a set of environmental cues. For plants, those cues may be the amount of light, temperature, and moisture. For tissues, these environmental cues may be hormones or other signals near the cells.

**Stem Cells**

In most towns, there are probably a few people who could reproduce the structure and function of the town’s government if the town hall burned down. In the same way, many tissues have a few cells with enough knowledge to reproduce the whole tissue. These “smart” cells are **tissue-specific stem cells**. Unlike other specialized cells, stem cells are immature; that is, they are not very differentiated. Their job is to provide new cells for the tissue. When the tissue needs a specialized cell, a stem cell reproduces, dividing into two “daughter” cells. One daughter is a specialized cell, while the other is another stem cell.

In a sense, tissue-specific stem cells have the blueprint for that tissue. They make sure that cells grow in the right places in the biomaterial. Thus, tissue-specific stem cells are very valuable in tissue engineering because they can repair the same type of tissue.

The stem cells in bone marrow are even more remarkable. The bone marrow is the soft material inside our bones that makes new blood cells and produces the numerous types of immune cells that help us fight disease. Some of the bone marrow’s stem cells produce all the types of blood cells to meet the body’s needs. Other bone marrow cells can produce cells for fat, cartilage, muscles, tendons, and other tissues! In the future, tissue engineers may be able to coax a bone marrow stem cell into producing the differentiated cells and tissue structure to repair damage elsewhere in the body.

**A Database on Development**

Developmental biology studies the timing and sequence of tissue development. Changes in tissues occur over the course of an individual’s life. Computers are helping us track changes in a tissue’s shape and function over time. This information will help scientists create more life-like tissues.
Skin: Your Body's Shield

Your skin protects you from invading organisms, controls your body temperature, contains touch and pressure sensors that alert you to danger, and keeps your organs on the inside! In the course of your life, your skin has probably healed from some pretty painful cuts, scrapes, and burns. But sometimes skin can become so badly injured that it cannot grow back. In that case, a person can die from infections. The only hope is to replace the lost skin with new skin.

Traditionally, doctors remove healthy skin from one part of the patient’s body to “graft” or place on the damaged area. However, this skin graft method damages the body where the skin is removed, and sometimes there is not enough healthy skin to use. The body routinely rejects grafts from other people.

Developing a way to save burn victims and others has been one of the first goals of tissue engineering – and an early success. One method uses cells called fibroblasts from the deep layer of skin called the dermis.

Unlike the muscle cells you will read about in the next article, fibroblast cells divide readily to reproduce. Scientists create sheets of biomaterial scaffolding containing collagen, a protein naturally found in skin. Inside this scaffold, the fibroblasts grow into a layer of dermis. Doctors place this layer on the patient’s wounded surface, where it begins to establish a blood supply and live on its own. To create the epidermis, scientists grow keratinocytes cells that make up this thinner, outer layer of skin. When these cells form a thin sheet, they can be placed on top of the dermis layer.

Bones: Pillars of the Body

Broken bones usually heal, but sometimes not perfectly. Cancers and other diseases of the bones can destroy them, and many people are born with missing or deformed bones.

Bones have several components: minerals to give them hardness; proteins to give them strength; blood vessels to nourish them; and special cells that build and remodel them. These special cells are osteoblasts and osteoclasts.

Osteoblasts build bone material to make it thicker and stronger at certain sites. Osteoclasts dissolve bone. Together, they form a team that grows and remodels bones throughout life as you grow taller, stronger, heavier, and older.
Tissue engineers can use the osteoblasts to grow new bones. They place these bone-grower cells in a biomaterial scaffolding with the mineral component of bone. The cells use this structure for support while they produce the proteins and minerals to grow new bones. Placing growth factors in key areas of the scaffold helps shape the bone growth. In some cases, the scaffold is placed right on the bone defect in the patient, and new bone tissue grows into the scaffolding.

Tissue engineers hope to be able to design a bone to match the shape of an individual patient. Computers will help by layering cells and biomaterials in two dimensions at a time, building towards the complex three dimensional structure of a real bone.

**Cartilage: Shock Absorbers**

Many an athlete has been brought down by damaged cartilage. Cartilage is the cushioning tissue in our joints and knuckles, and it gives shape to our noses and ears. It has a texture like a cake of dry soap. When lubricated by joint fluids, it provides a slippery surface for the bones in our joints.

Since cartilage does not require a blood supply, it can survive on the nutrients from nearby tissues and from joint fluids. Establishing a blood supply remains a major hurdle for engineering more complex tissues.

When cartilage in a joint is damaged, the bones grind together, causing pain and damaging bones. Repairing joints can restore athletes to the playing fields and keep people off crutches and out of wheelchairs.

Such repairs have become fairly common these past decades. They help keep people active, but they are not perfect. A standard replacement part is made of metal or plastic molded to the shape of a normal hip joint. This solid material permanently replaces the entire joint. It cannot grow and remodel itself as the person grows and ages. After ten or twenty years, it often needs to be replaced again.

Tissue engineers are working to overcome these problems. They are developing a hip replacement using new biomaterials that can become part of the living, growing, changing body. It begins as a porous scaffold with space for the cartilage cells to grow. These cells gradually replace the biomaterial, leaving a "living" joint that can grow and change along with the body.

Another method is already being introduced. Doctors inject cartilage into a patient’s injured joint. There, the cells rejoin the damaged cartilage and become anchored to the surrounding tissue.

In the future, doctors will be able to use cartilage to rebuild a badly injured nose, cheek bone, or jaw. They hope to be able to inject cartilage tissue with a soft biomaterial scaffold that gels at body temperature to take a desired shape. After several months, the cartilage cells will replace that scaffold, forming a cartilage tissue with the same contour. Thus, the face will be rebuilt from the inside, without the pain, expense, and difficulties of plastic surgery.
Heart: Power Supply of the Bloodstream
The heart is an incredibly complex organ. In addition to its four chambers, it has a muscular wall, blood vessels, an electrical system, and large valves to direct the flow of blood between chambers. Fortunately, when a heart goes bad, we can replace heart valves and blood vessels, and sometimes the heart itself. However, the supply of healthy hearts for heart transplants is very limited, and a patient’s immune system often rejects the “foreign” organ.

Tissue Engineering may eventually overcome problems of shortage and rejection. Tissue engineers can already grow heart valves using biomaterials and human cells. They are working on ways to grow blood vessels and to strengthen the heart walls by transferring muscle cells from the limbs to the heart. (See the Profile on page 14.) One day they may be able to engineer an entire three-dimensional heart shaped muscle to replace the heart itself.

Liver: Setting the Body Right
Perhaps less famous than the heart, the liver is equally complex and vital. It creates proteins, protects against infection, removes toxins from the blood, and helps digest food. To do all these tasks, it has two blood supplies, ductwork for the removal of bile, and a unique tissue structure that allows it to process body fluids.

When a liver becomes badly damaged by disease or alcohol abuse, the patient will die unless a rare liver transplant is available. To help people waiting for a transplant, tissue engineers created a partial replacement liver. This structure contains liver tissue in a biomaterial casing. It is attached to a patient’s arteries and veins but remains outside the...
Spare Parts: Who Gets Them?

In the future, we may have the technology to make replacement tissues and organs for any individual. Clearly, these replacement parts could solve many life-threatening medical problems. But, like most scientific advances, these benefits may be complicated by difficult choices. Here are some of the concerns scientists have:

1) Availability: Who will get a replacement part if there are not enough resources to make one for everybody who needs one? Will young people be favored over old? People who have taken care of their bodies over people who abused them with cigarettes and alcohol? Who will set the priorities?

2) Cost: Engineered tissues will be expensive. Will only wealthy people be able to afford them? Should health insurance companies cover them for everybody? Will we begin to think we have a “right” to new tissues?

3) Age: How late in life should we keep replacing organs? Should there be a cutoff age? Should we keep trying to rebuild worn-out bodies?

Muscles: Moving Through Life

Many diseases such as muscular dystrophy cause muscles to degenerate. In some cases, people lose strength in the large muscles, such as those that move arms, legs, and the head. In advanced cases, people also lose the involuntary muscles that allow them to eat, breathe, and digest food. They need respirators and feeding tubes to survive. Thus, learning to repair and strengthen muscles could prevent a lot of human misery. Tissue engineers are hoping to do just that.

When muscle cells grow in a laboratory culture, they join together to become fibers. If the culture is “stretched” the way real muscles stretch, these fibers form very thin muscle-like tissues. Tissue engineers can coax the muscles to form a predictable structure. To do so, they use laser patterning techniques similar to those used to make printed circuit boards for electronics. They “print” a pattern on the biomaterial where cells will attach. The patterns imitate the structure of a particular type of muscle.

The next challenge for tissue engineers is to find a way to make blood vessels and nerve cells grow into muscles. Then, these muscles may be used to treat paralysis or muscular weakness.

If you could go in for a “tissue tune up” every twenty years, would you still try to take care of your body, or would you just wait for a tissue “upgrade?”

“Hearts!! No, No, …We wanted livers!”

These are magnified images of myoblasts patterned on biomaterials. The image on left shows very narrow adhesive lines and the image on right shows wider adhesive patterns.

Tahsin Oguz Acarturk, MD, Paul A. DiMilla, Ph.D, and Patti Petrosko, MS
To build a house, we have to know how it will look on the outside, what its internal structure will be, and the stages in which different parts will be built. Tissue engineers need to know similar things to build a new tissue: how a real tissue looks inside and out, how it works with other tissues, and how it develops and grows.

A Study in Tissues
When people first studied tissues, they were fascinated by the differences in texture, color, form, and function. People from the past would be amazed at how completely we can now “see” tissues. The National Library of Medicine’s “Visible Human” project shows a slice by slice view of a body from the inside. (See graphic on page 4.) Each view is digitized on the computer, so scientists can pluck a “virtual” tissue from the body, turn it, and study its shape, texture, and organization. This ability will help tissue engineers manufacture artificial tissues.

The invention of the microscope allowed people to see that many individual cells are the building blocks of tissues and to observe how the structure of tissues affects their function. Today, new imaging methods provide glimpses of how tissues look in action in living animals and humans. These images provide the foundation for learning how to build replacement tissues that will function properly in the body.

The relatively new study of genes allows us to understand the cells within tissues on a genetic level. The international Human Genome Project has identified many genes responsible for the structure and function of tissues. Tissue engineers may be able to use this knowledge to change a diseased tissue by inserting a healthy gene in it, or to create customized replacement tissues.
If tissue engineering sounds “space age,” consider this. Scientists at NASA are growing tissues both aboard the Space Shuttle and at the Johnson Space Center in Houston. Why? Because earthbound scientists have found that, in some situations, gravity interferes with the way engineered tissues grow in the laboratory. They do not develop the proper shape of natural tissues. Tissues grown on the Space Shuttle or in the Space Center’s “microgravity bioreactor”— which is an incubator for growing cells without the influence of gravity — have a more natural structure. Eventually, these experiments may help us learn how to develop more natural-looking tissues in our natural gravitational environment. After all, our own tissues grow normally in gravity!
Doris Taylor's research may provide a long-awaited cure for a common type of heart disease called congestive heart failure. This heart failure often follows a heart attack, which scientists call acute myocardial infarction. Myocardial refers to the muscle (“myo”) of the heart (“cardia”), and infarction means damage from lack of blood (usually because the artery is clogged). After a heart attack, the damaged portion of the heart muscle dies, and the heart eventually fails. “The heart cannot repair the damaged muscle because its muscle cells cannot reproduce,” Doris explains. “You are born with all the heart cells you will ever have. Your heart grows because the cells become larger, not because they multiply.”

However, other muscles do have the ability to repair themselves because they contain cells called myoblasts, which can reproduce. Myoblasts are immature tissue-specific stem cells in muscles that can produce more specialized muscle cells when needed. “We are always damaging our skeletal muscles – the ones that move our bones – when we strain them or bump into things,” Doris continues. “When our skeletal muscles are damaged, they stimulate the myoblasts to reproduce and make more muscle cells to repair the damage.”

Doris asked herself, “Why don’t we take skeletal myoblasts and see if we can transplant them into the heart and get them to live there?” She hoped that the transplanted myoblasts might reproduce as they do in skeletal muscles and replace the damaged heart cells. Her laboratory experiments with animals show that the myoblasts do seem to help the heart muscle repair itself!

Every year in the United States, about half a million people have heart attacks. Many of them go on to develop heart failure – a leading cause of death in people over 65. Doris envisions the following scenario. “When someone comes into the emergency room with a heart attack, we take a tiny bit of muscle from their arm or leg and extract the myoblast cells. The ER doctors continue with their usual treatment, and we take the myoblasts to the lab to grow them in a culture. After a few weeks, when we have 10,000,000 or more myoblasts, we implant them into the patient’s damaged heart muscle, and that patient will soon have a repaired heart instead of one that is likely to fail again.”

The next area of research is to find ways to make the myoblasts more heart-like. “If we put them in the kind of extracellular matrix found in the heart, and then stretch them to simulate a beating heart, perhaps they will become more heart-like. For example, maybe they will form the kind of electrical connections that other heart cells have. That would be incredibly important!”
**Strong Bones/Weak Bones**

You have probably learned that calcium builds strong bones. Your bones actually start out fairly soft and flexible. The bone tissue develops around a scaffold made of elastic fibers and collagen, which is the soft, flexible material that also forms the scaffold of cartilage and skin. As bones develop, the bone cells deposit the mineral calcium in the scaffold. This calcium gives bones more strength, density, and mass.

Bones are dynamic tissues that are always losing and gaining calcium. As you grow old, your bones tend to lose more than they gain. To keep your bones strong, you need to add calcium to your bones throughout your life. Otherwise, your bones will lose their density and mass because they are losing calcium. This loss of calcium causes broken bones, bent backs, and shrinking height. Have you ever seen a stooped over old person or someone with a “dowager’s hump?” Their vertebrae are so demineralized that they collapse.

In this activity, you will see first hand what happens when bones become demineralized. To understand what happens, you should know that acids can leach (dissolve) minerals out of other substances. Acid rain can leach mineral nutrients out of soil, and acidic water can leach dangerous metals such as lead and copper out of pipes. In the same way, acids can leach calcium out of bones.

**Materials**

- Three cooked chicken thigh bones
- Two 250 mL beakers
- Vinegar
- Distilled water

**Procedure**

1. Examine the thigh bones and note the different kinds of tissues you see. Draw a diagram of the bone and label the tissues.
2. Place a bone in a beaker and pour in vinegar to cover.
3. Place a second bone in a beaker and pour in distilled water.
4. Allow the bones to soak for three days.
5. After three days, remove the bones and rinse under running water.
6. After the bones have dried, place them next to the third, untreated chicken bone. Compare the way the three bones look and record your observations.
7. Now test and compare the flexibility and rigidity of the three bones by trying to bend and twist them. Record your comparisons.

**Conclusions**

1. Did either of the two liquids affect the flexibility and strength of the bone?
2. Which of the two liquids do you think is acidic?
3. Which of the soaked bones shows what the bone’s scaffold is like before it becomes mineralized?

**Extensions**

1. Describe how the bone structures of a new born baby, a teenager, and an elderly person differ.
2. The loss of bone mass in older adults is called osteoporosis, and it is a major health issue for the elderly. Find out more about why people lose their bone mass, what you can do to prevent it happening to you, and why you should start now!
Dear Students:

Biotechnology and the rapid advances of science are in the news often because they are providing new opportunities for improving human and animal health, agriculture, and the restoration of damaged environments. This issue of Your World/Our World is designed to allow you to explore how biotechnology will influence your life and your world by introducing you to the new area of tissue engineering. Research in this area is underway to discover how tissues can be restored, maintained, or replaced by engineering or creating new tissues.

We hope that greater understanding of emerging scientific areas will encourage you to continue to study science and mathematics. We also welcome your selection of biotechnology as a career and your participation as a co-discover of tomorrow's science and technology.

Sincerely,

Jeff Davidson
Executive Director, Pennsylvania Biotechnology Association

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**Organ** | **Function**
---|---
Brain | Controls sensation, muscles, thought
Heart | Pumps blood throughout the body
Liver | Makes proteins, assists digestion of fat, removes toxins
Intestine | Digests food
Muscle | Moves the body
Bones | Supports body structure, makes blood cells (marrow)
Skin | Protects body, provides touch sensation, controls temperature
Cartilage | Forms joints, ears, and nose

**References**

Websites:


Other Issues of Your World/Our World

- Exploring the Human Genome (Vol. 5, #2):
  The Human Genome Project
- Investigating the Brain (Vol. 6, #1):
  The structure and function of the brain.

**Organ Function**
- Brain: Controls sensation, muscles, thought
- Heart: Pumps blood throughout the body
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**Websites**