The Science of Stretch

The study of connective tissue is shedding light on pain and providing new explanations for alternative medicine.

By Helene M. Langevin | May 1, 2013

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It joins your thigh to your calf; your hand to your arm; your breastbone to your clavicle. As you move, it allows your muscles to glide past one another. It acts like a net suspending your organs and a high-tech adhesive holding your cells in place while relaying messages between them. Connective tissue is one of the most integral components of the human machine. Indeed, one could draw a line between any two points of the body via a path of connective tissue. This network is so extensive and ubiquitous that if we were to lose every organ, muscle, bone, nerve, and blood vessel in our bodies, we would still maintain the same shape: our “connective-tissue body.”

Despite increasing evidence of its role in chronic pain and other diseases, connective tissue is not very well studied. I arrived at researching connective tissue by a circuitous route. Working as a clinical endocrinologist, I would see patients suffering from chronic pain, and quickly became frustrated with the treatment options I could offer—usually some combination of physical therapy and analgesics, which often were not very effective. Some of my patients would ask about trying acupuncture. But, having done research in neuroscience and being firmly rooted in the practice of Western medicine, I was skeptical. Eventually, I decided to learn more, if only to be able to respond to patient questions more intelligently.

In 1986, I took evening classes at the Tri-State Institute of Traditional Chinese Acupuncture in Stamford, Connecticut (now the Tri-State College of Acupuncture in New York City), which offers hands-on experience in acupuncture. The teacher described how to twirl the inserted acupuncture needles just enough to elicit a particular sensation in the patient, usually described as an ache in the area surrounding the needle, which can radiate some distance away from it. I was told that the acupuncturist is supposed to feel tightness or tugging on the needle, akin to when a fish gets caught on a hook. When I felt that tug myself, I became curious about the physical mechanism that was causing it. The teachers explained it as muscle contracting around the needle, but I could feel it in locations, such as the wrist, where there was no muscle at all. The needles had to be interacting with connective tissue.

A decade later, after I had moved to the then Department of Neurology at the University of Vermont (UVM) College of Medicine in Burlington, I had the opportunity to begin research on the acupuncture “needle grasp.” Here was a physiological phenomenon that one could feel with one’s fingers, but which had no obvious biological explanation. I started collaborating with Martin Krag, an orthopedic surgeon at UVM who had some experience testing alternative-medicine approaches using scientific methods. The logical first step was to quantify the tugging response to acupuncture needling. With the help of David Churchill, a biomedical engineer in the Orthopedic Department at UVM who designed a robotic acupuncture-needling instrument, we began measuring the force needed to pull out the needles in a reproducible manner from 16 different points on the body. We measured the “pullout force” in 60 human subjects and found that it did indeed increase after needle rotation, at times so dramatically that it exceeded the capacity of our 500 g load-measurement device.[1]

We then tested the possible mechanisms that could cause this phenomenon, starting with simple experiments in which we inserted and rotated a needle in a piece of rat abdominal wall. What we saw under the microscope was quite striking: when acupuncture needles were rotated, the loose connective tissue under the skin became mechanically attached to the needle. Even a small amount of rotation caused the connective tissue to wrap around the needle, like spaghetti winding around a fork.[2] This winding caused the surrounding connective tissue to become stretched as it was pulled by the needle’s motion. Using ultrasound, we confirmed that the same phenomenon occurs in live tissue.[3]

In the years that followed, I became part of a small but growing community of scientists who were joining the ranks of molecular and physiological researchers dedicated to studying this neglected tissue. Connective tissue has been relegated to the role of passive viscoelastic material in traditional biomechanical models, but researchers are now beginning to demonstrate just how many systems of the body may be affected by mechanical changes in connective tissue, and some of these findings are beginning to inform clinical practice.

A growing field

Connective tissue is something of an orphan child in medicine: although it is an integral part of the musculoskeletal system, connective tissue is basically absent from orthopedic textbooks, which deal principally with bones, cartilage, and muscles. Orthopedic interest is almost exclusively restricted to the “specialized” connective tissues such as tendons and ligaments, which connect bone to muscles and to other bones, respectively. Nonspecialized connective tissues, which form what’s known as the fasciae and envelop all muscles, nerves, bones, and blood vessels, are typically allotted a short paragraph in current textbooks, if mentioned at all.

However, interest in the field has been growing. One area that has attracted many researchers at the cellular level is the study of mechanotransduction: how the integrin family of adhesion molecules forms a physical and informational link between the extracellular matrix and the interior of cells. Through these cell-matrix connections, cells sense forces and transform these mechanical signals into cellular responses such as the activation or deactivation of signaling molecules, translocation of transcription factors into the nucleus, and ultimately, changes in gene expression.[4] In addition, substantial evidence supports the notion that mechanical signals can be transmitted directly through the cytoskeleton into the interior of the nucleus. (See "Full Speed Ahead," *The Scientist*, December 2009.)

Some of the most well-established work in this field has involved the study of fibroblasts—the cells that are responsible for synthesizing all the proteins that make up the extracellular matrix. These cells reside within the matrix they create, responding to mechanical stimulation by regulating the amount of collagen and other matrix proteins produced, and by secreting matrix-degrading enzymes in response to chronic changes in tissue forces. Such changes can be induced by repetitive motion and are thought to be an important factor in work-related musculoskeletal injuries such as tendinitis of the shoulder or wrist.[5]

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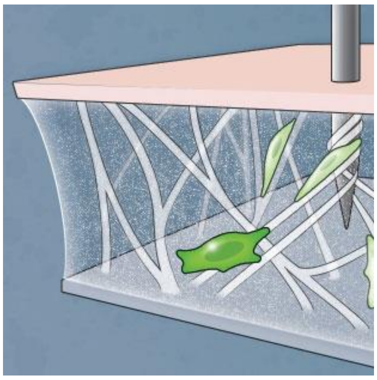
Fibroblasts also play a major role in the response to acute injury, particularly when they transform into myofibroblasts. Before the availability of surgery and surgical sutures, gaping wounds needed a powerful mechanism in order to pull shut and heal. Myofibroblasts serve this function by secreting large amounts of collagen and expressing α-smooth muscle actin protein, which make the cells contractile.[6] Then, by exerting tension on the collagen matrix, these cells pull the edges of the wound together. Myofibroblasts normally die once this job is done and a stable scar has formed. However, during chronic inflammation, myofibroblasts can drive an excessive deposition of collagen, and the increased tissue tension can result in the development of tissue contractures that restrict full range of motion. This response is also thought to play a role in the development of some types of tissue fibroses and cancer. Indeed, fibrotic, or scarred tissues, become stiffer, and cancer cells have been shown to spread more easily on fibrotic matrices.[7]



WHEN CONNECTIVE TISSUE STRETCHES
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Although much of the work in this area to date has been performed in cell culture, rather than in whole tissue, some of this basic research is beginning to inform clinical research and practice, especially in the area of chronic musculoskeletal pain, including low-back pain. One of the reasons that low-back pain is so difficult to manage is that large numbers of patients have no detectable abnormalities of the spine and associated tissues, and the source of their pain is unknown. Some groups have begun to investigate the possibility that the pain is arising from the nonspecialized connective tissues on either side of the spine.

Indeed, researchers at the University of Heidelberg found in 2008 that connective tissues contain sensory nerve endings that can transmit pain when these tissues are stretched in the presence of inflammation.[8] Until then, it had not been clear whether connective tissue had its own sensory nerve supply capable of generating sensations. Subsequently, ultrasound studies in my laboratory demonstrated that the connective tissues that surround the muscles of the back are, on average, thicker in people with chronic low back pain.[9] Normally, these connective tissues are composed of alternating layers of tightly woven dense fibers that can bear substantial loads, and loose areolar tissue, which contains large quantities of water and allows the adjacent dense layers to glide past one another. In addition to having thicker connective tissue overall, people with low-back pain show a decreased gliding motion of dense layers, suggesting that a fibrotic process could cause the decreased mobility.

Connecting the dots

TISSUE TENTING: A twisted acupuncture needle creates a localized stretch by gripping the underlying connective tissue. This effect can be observed as a “tenting” of the skin as the needle is pulled out.
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Despite these recent advances, the overwhelming majority of research on connective tissue still involves cells grown in culture dishes. And recent studies suggest that, especially for fibroblasts, the mechanical behavior of cells may be quite different when cells are grown on 2-D surfaces compared to cell behavior in a 3-D environment that is more similar to that of whole tissue, such as a thick collagen gel. For example, it is becoming apparent that the ubiquitous intracellular “stress fibers” characteristic of fibroblasts grown on 2-D surfaces are not present in fibroblasts grown in 3-D-culture environments or in whole tissue, and that these fibers may in fact be an artifact of cell culture, rather than a phenomenon that has physiological meaning. The fact that the study of fibroblasts in whole tissue is lagging far behind that of fibroblasts in vitro, combined with the general lack of attention to nonspecialized connective tissue at the whole-body level, has limited the understanding of natural connective-tissue function.

I began my research into connective tissue on the whole-animal level, but quickly began to investigate the cellular components involved in the winding response to acupuncture needles. After dissecting some of the tissue we had manipulated, we saw that the fibroblasts residing in the connective tissue as far as several centimeters away from the needle began to reorganize their internal cytoskeleton and change shape, becoming large and flat. We also found that the same reorganization response could be elicited by simply stretching a piece of connective tissue between two grips and holding the tissue in the stretched position for about 30 minutes, or even stretching an anesthetized mouse by bending its body to one side.[10] Interestingly, 30 minutes is typically the amount of time that needles are left in place during an acupuncture treatment. Furthermore, if one lets go of the needle after rotating it, the needle does not unwind right away. Thus, the “whorl” of connective tissue remains intact as long as the needle remains under the skin, causing the tissue to be stretched for a prolonged period.

Ongoing studies in my lab are addressing why the fibroblasts change shape in response to sustained stretching. So far we have found that the changes are associated with a large-scale relaxation of the connective tissue. We also saw that the fibroblasts initiated a specific Rho-dependent cytoskeletal reorganization that was required for the tissue to fully relax. Rho is an intracellular signaling molecule known to play a role in cell motility and the remodeling of cell-surface proteins that connect the fibroblast to its surrounding matrix. The molecule’s involvement in fibroblast shape change suggested that the cells are able to reduce the tissue tension by adjusting how strongly and where they are gripping the surrounding connective tissue or muscle. (See illustration above.) In addition, we found that the shape change is also associated with a sustained release of ATP from the fibroblast.[11] Within the cell, ATP acts as fuel, but outside of the membrane, ATP can function as a signaling molecule. Extracellular ATP can be converted to other purines such as adenosine, which can act as a local analgesic, thus providing a possible cellular and physiological mechanism to explain the pain relief experienced by some acupuncture patients.[12] (See “Puncturing the Myth,” *The Scientist*, September 2011.)

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The possibility that connective tissue dynamically regulates its level of tension is intriguing, as it could dampen fluctuations in tissue tension. Connective tissue surrounds nerves, blood vessels, and lymphatics, and reducing changes in tissue tension could affect how these structures function. Importantly, fibroblast cytoskeletal reorganization is a rather slow process, taking several minutes, and therefore would occur in response to sustained changes in tissue length such as changes in posture and body positions. Remarkably little is known about the effects of static tissue stretching, though repetitive, cyclical stretching has been extensively studied because of its relevance to breathing, walking, and cardiovascular pulsations. Acupuncture-needle manipulation results in sustained stretching, and therefore constitutes a useful tool that can be used to study this biomechanical function.

In contrast to the general neglect of connective tissue in the conventional medical and scientific fields, “alternative-medicine” researchers, and especially clinical practitioners, have for many years recognized the potential importance of connective tissue in health and disease. In conventional physical therapy, stretching of surgical scars and joint tissue that has contracted and stiffened after prolonged immobilization is widely believed to cause remodeling of connective tissue. Alternative therapies such as myofascial release and Rolfing focus on stretching as a treatment modality for musculoskeletal pain, even in the absence of an obvious past injury or scarring. Indeed, a variety of alternative manual and movement-based therapies work under the collective assumption that connective-tissue pathology lies at the source of musculoskeletal pain, and that this can be ameliorated with manual treatments.

Connection to acupuncture meridians

The mysterious "acupuncture meridians," defined as lines or tracks connecting acupuncture points, also may be related to connective tissue, as they seem to be preferentially located along connective-tissue planes between muscles, or between muscle and bone. We have found that more than 80 percent of acupuncture points in the arm are located along connective-tissue planes.[13] This makes sense, since loose connective tissue houses blood vessels and nerves, suggesting that mechanical stimulation of connective tissue generated by needle manipulation could transmit a mechanical signal to sensory nerves, as well as intrinsic sensory afferents directly innervating connective tissue.

Clearly, connective tissue needs more attention. A simple PubMed search illustrates this problem, as specific subject headings for "nonspecialized connective tissue" do not exist. By default, alternative medicine has become a motivating force in connective-tissue research and clinical practice. This is an example of an area in which the combination of conventional and alternative medicine, typically referred to as "integrative medicine," should be understood in a broader sense as integration within medicine itself, inspired by alternative-medicine concepts. The growth and maturation of the field of connective-tissue research will no doubt benefit from exciting new developments resulting from this integration.

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Tags

Physiology or Medicine, physiology, pain, ligaments, fibroblasts, extracellular matrix, connective tissue, collagen, cancer research and acupuncture