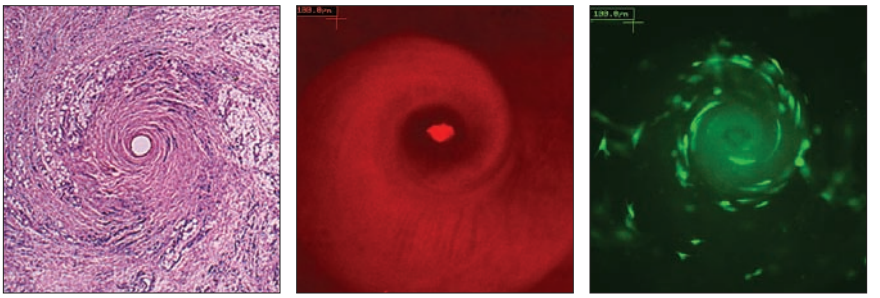
Acupuncture Tissue Mechanisms

Acupuncture is a centuries-old therapeutic technique whose biological mechanisms have largely eluded scientists. Because it can act rapidly over large distances, is effective at relieving pain, and can induce changes in heart rate, blood flow, and gastrointestinal responses, most studies have focused on possible neural mechanisms. However, studies by Langevin at the University of Vermont are showing that cellular and molecular mechanisms initiated in the local tissue surrounding the acupuncture needle may play a more significant role in the therapeutic effects than previously realized.



LEFT PANEL FROM LANGEVIN ET AL: *FASEB J* 15(12):2275-2282, OCT 2001; WITH PERMISSION.

Acupuncture needle rotation causes the randomly oriented collagen network to wind around the needle, with resident cells following the fiber alignment. *Left,* Optical microscopy shows a spiral winding pattern around the needle in rat subcutaneous tissue (hematoxylin-eosin stain). *Center,* A similar winding pattern is observed in a fibroblast-populated collagen tissue equivalent, visualized by crosslinking the collagen with genipin and viewing with fluorescence confocal microscopy. *Right,* In the same sample, fibroblasts expressing green fluorescent protein mimic the collagen realignment. (GFP-expressing fibroblasts a gift of Rutgers' W.M. Keck Center for Collaborative Neuroscience.)

Acupuncture is performed by inserting fine needles through the skin into the subcutaneous tissue at specific points on the body known as *acupuncture points*. These points lie at well-defined locations, determined in ancient times by their ability to evoke a desired therapeutic response. The recent discovery that many of these sites coincide with inter- and intramuscular connective tissue planes originating under the skin surface has suggested the connective tissue may be a key mediator of the therapeutic response.

Further evidence for this idea has come from measurements of the biomechanical response to acupuncture. An important component of the clinical acupuncture technique is manipulation of the needle in the tissue by the practitioner. This manipulation typically takes the form of rotational or up-and-down movements. When performed effectively, needle manipulation leads to a perceptible resistance to needle movement—i.e., the practitioner must exert greater force to rotate or oscillate the needle after manipulation than before.

Use of a computer-controlled needling instrument equipped with force sensors to monitor the force on the needle during acupuncture treatment has shown that the force needed to pull the needle out of tissue (pullout force) following rotational manipulation is on the order of 1 N, 167% greater than the pullout force when the needle is simply inserted and removed without manipulation. During needle rotation, the torque on the needle increases monotonically to values on the order of 1 mN-m.

The increase in torque and pullout force with needle rotation appears to result from the winding of connective tissue around the needle. Histologic study and ultrasound microscopy of rat abdominal wall tissue following acupuncture needling reveal the formation of an obvious spiral pattern in the tissue surrounding the needle in the plane perpendicular to the needle axis. The only significant structural change seen through the thickness of the tissue, parallel to the needle axis, is a marked thickening of the subcutaneous connective tissue. This thickening occurs over a radial distance from the needle corresponding to the spiral pattern.

Staining for collagen and fibroblasts, the primary components of connective tissue, in the thickened region shows the realignment of collagen fibrils consistent with the spiral pattern and corresponding changes in fibroblast shape, alignment, and F-actin distribution.

These observations, taken together, suggest a mechanical model in which the connective tissue fibrils attach to and wind around the rotating acupuncture needle. As the winding proceeds, a three-dimensional connective tissue whorl forms, deforming the tissue and increasing the mechanical stress in the area surrounding the needle. The mechanical stress leads to phenotypic responses by fibroblasts.

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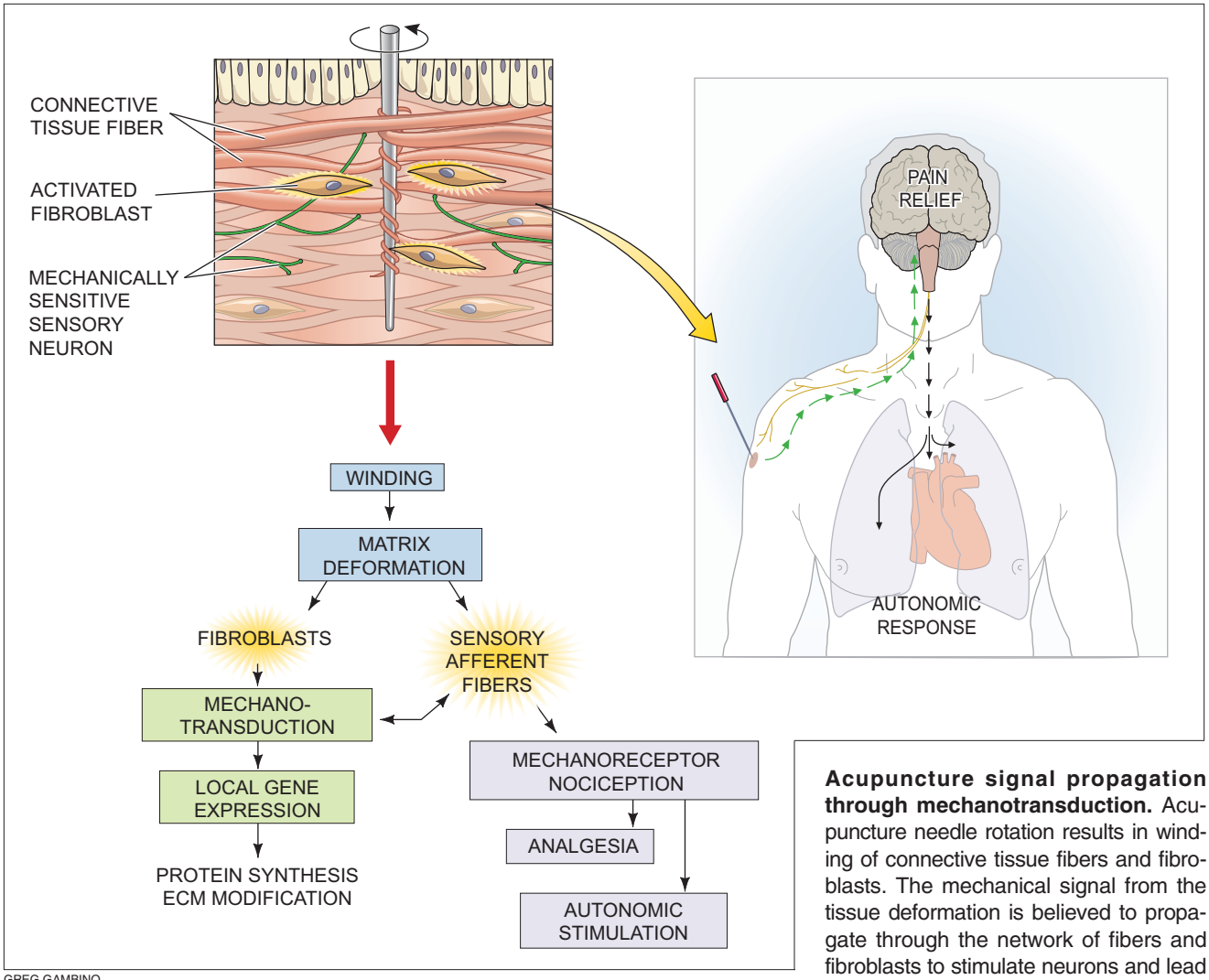

GREG GAMBINO

Acupuncture signal propagation through mechanotransduction. Acupuncture needle rotation results in winding of connective tissue fibers and fibroblasts. The mechanical signal from the tissue deformation is believed to propagate through the network of fibers and fibroblasts to stimulate neurons and lead to centrally mediated physiologic responses, as well as locally mediated cellular responses.

Mechanical deformation and cell phenotypic responses in other systems have been shown to lead to a variety of cellular events, including intracellular signaling, changes in gene expression, and synthesis of proteins such as those of the extracellular matrix, enzymes, growth factors, and vasoactive substances, as well as stimulation of mechanically sensitive neurons.

Thus, it has been proposed that acupuncture initiates a mechanotransduction response that propagates through the tissue both spatially and temporally via a series of events induced by the mechanical stimulus of needle manipulation.

This mechanotransduction hypothesis of acupuncture has grown out of human and animal studies done in the laboratory of Dr. Langevin in Vermont. To evaluate the cellular and molecular events initiated in connective tissue by acupuncture needling, Drs. Buettner and Shreiber at Rutgers are using tissue engineering techniques that allow systematic elucidation of critical features of the tissue environment and mechanotransduction response.

In the past several decades, tissue equivalents comprising isolated tissue cells cultured in biopolymer gels have become an increasingly important vehicle for examining macroscopic, microscopic, and submicroscopic phenomena associated with mechanotransduction. These three-dimensional tissue culture systems have been used with single cell or coculture populations to simulate a plethora of tissue systems, including dermal, vascular, pulmonary, bone, hepatic, and cardiac tissues.

The three-dimensional architecture allows physiologically relevant mechanostructural signaling via cell–cell and cell–matrix connections, while preserving control over culture conditions and the ability to dynamically monitor the behavior of cells and tissue reorganization. Similarly, the control over cellular, matrix, and media composition and the ability to record forces and deformations with optics and/or instrumentation have greatly facilitated the development of advanced multiphase models of tissue and cell mechanics and interstitial flow.

These models have dramatically improved our understanding of the biophysics of physiologic phenomena such as cell migration and tissue contraction involved in tissue morphogenesis and wound healing, and they have been instrumental in the continued development of tissue-engineered therapies in regenerative and reparative medicine, such as bioartifical skin, small-caliber vascular grafts, and heart valves.

Even the simplest tissue equivalent systems—for instance, fibroblasts or smooth muscle cells uniformly dispersed in a type I collagen gel—can provide a fascinating window into the biology and biophysics of cell-tissue interactions. These cells will attach to the fibrillar collagen via integrins and contract the gel via intracellular forces derived from actin and myosin cytoskeletal elements. Varying the mechanics of the tissue equivalent by adding boundary conditions to restrict compaction or modulating the stiffness of the gel alters the force balance that exists as cells pull on the matrix through focal adhesions, and it can result in morphologic and phenotypic changes in cell behavior and potentially tissue organization.

For example, adding mechanical stress by restricting gel compaction drives fibroblasts to be more contractile and significantly changes the expression of a variety of genes, including those for integrins, cytokines, growth factors, and extracellular matrix molecules. Advanced assays to study specific aspects of cell behavior governed by cell-tissue interactions during wound healing, such as cell migration, chemotaxis, and wound contraction, are then developed by systematically introducing soluble factors typically present in a wound environment to this culture.

Similarly, tissue equivalents provide excellent vehicles for examining the influence of externally derived forces on cell and tissue behavior, such as those experienced in vivo during various functions of tissue (distension of blood vessels), organ (heart contraction), and organism (mechanical loading of tendons, ligaments, and cartilage during movement).

For example, imposing cyclic strain on smooth muscle cells cultured in a collagen gel significantly affects matrix and metalloproteinase gene regulation and results in significant matrix turnover and stiffer, stronger constructs, as well as increased cell contractility. By manipulating culture conditions via genetic modification of cells, defined medium and matrix components, and/or mechanical loads, great advances can be made in isolating specific mechanisms and pathways involved in reparative and pathogenic phenomena.

We are applying these same principles in the development of three-dimensional tissue equivalent models to investigate the mechanisms of acupuncture. Fibroblast-populated collagen gels subjected to acupuncture needling in vitro demonstrate fibril winding, tissue thickening, cell alignment, and cytoskeletal reorganization consistent with the observations in vivo and in tissue explant studies. Preliminary finite element modeling predicts a highly localized strain field within a few millimeters of the needle, and force measurements demonstrate increased torque resistance and pullout strength, which is again consistent with the findings of Langevin.

By working with tissue equivalent models in an in vitro setting, we can generate assays with conditions to evaluate specific mechanisms of mechanical signal propagation. For example, using rapid prototyping, we are developing assay chambers with geometries that mimic perimuscular connective tissue planes to investigate their role in needle coupling during acupuncture.

We are also expanding this assay platform to include other cell types potentially involved in mediating the response to acupuncture, such as neurons, vascular and lymphatic endothelium, and inflammatory cells, to examine heterotypic cell interactions. As with other three-dimensional in vitro assays, our models of acupuncture enable the real-time dynamic assessment of cell and tissue behavior with advanced microscopy.

Acupuncture represents a fascinating scientific and biomedical question. Because of the range of clinical conditions potentially treatable by acupuncture, a successful study of the mechanisms underlying this therapy has far-reaching implications. Perhaps the most intriguing aspect of the research is the consideration of acupuncture as a microcosm of how simple mechanical forces can assist in tissue repair and regeneration and can induce system-level changes.

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