i-Needle: Detecting the Biological Mechanisms of Acupuncture

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A long standing obstacle to the (full) integration and acceptance of acupuncture in conventional medicine lies in the difficulty of reconciling traditionally defined categories (acupoints, meridians, and energy flow or *qi*) with anatomical structures and biochemical pathways. Additionally, a unified scientific theory to explain the diverse effects of acupuncture (pain control, cellular regeneration, immunomodulation) is lacking, despite important advances in the association of purinergic signaling with the effects of acupuncture on pain control. As new technologies simultaneously offer enhanced capacities to explore breadth (using 'omics) and depth (using nanobiochips) of biochemical events, we propose the innovative conjugation of these approaches into an intelligent needle (*i*-needle) as a means to overcome the abovementioned limitations.

Acupuncture is being widely debated in the medical community as a potential alternative or complementary treatment for many diseases (*1*). There are numerous challenges to achieving a consensus over the use of acupuncture in a medical environment, including: filling the gap in knowledge about the underlying molecular mechanisms of acupuncture, and (re)interpreting traditional categories (such as acupoints, meridians, and *qi*) and therapeutic indications within an evidence-based medicine framework. Important questions aimed at increasing our understanding of the molecular effects of needle stimulation have been posed, mostly regarding pain control (*2*), cellular regeneration (*3*), and immunomodulation (*4*), with remarkable work done as to the correlation of pain control with purinergic signaling (*5, 6*). Using 'omics-based technology and network representations, researchers have successfully mapped the molecular underpinnings of traditional categories (*7*). More generally, the *holistic* method used in acupuncture, which has long been difficult to reconcile with the scientific reductionist viewpoint, has recently been found to be compatible with a systems biology approach (*8*).

'Omics-based techniques are diverse and allow for the screening of targets from nucleic acids (DNA-sequencing, RNA-sequencing) to proteins and metabolites (mass spectrometry/liquid chromatography, nuclear magnetic resonance) and their heterogeneous interactions (chromatin immunoprecipitation-sequencing), to name just the major technologies. Recently, whole new areas of exploration have been opened with metagenomics and metatranscriptomics where the host-microbiome relationship can be analyzed systemically and in situ. Further, rapidly decreasing costs are permitting researchers to prefigure relatively high spatial (different body regions and tissues) and temporal resolution. Here, we propose to

integrate such highly resolved molecular, temporal, and spatial data to reveal the molecular signaling pathways that flow from the tip of the needle to the disease/injury site.

Understanding the biochemical signaling pathway that the mechanical rotation of an acupuncture needle sets into motion (*9*) is an important starting point. Mechanosensing and mechanotransduction are widespread in biology with well-assessed relevance in embryonic development, i.e., type 1 epithelial-mesenchymal transition (EMT) (*10*). Their roles, however, have not been well explored under the broader definition of EMT—which includes events such as wound healing (type 2 EMT) and cancer (type 3 EMT)—despite promising results when mechanical stimulation is locally applied (*11*). Acupuncture needle stimulation (*9*) and low level laser therapy (*12*) are among the triggers that have been shown to initiate a series of synergistic events, including calcium waves, ATP fluxes (purinergic signaling), and changes in reactive oxygen and nitrogen species concentration, known to initiate healing (*13, 14*). The homeostatic effects of type 2 EMT include local changes in purinergic signaling, inflammation control, regeneration, and remodeling at the site of injury. By contrast, acupuncture is recommended for systemic diseases like rheumatoid arthritis (*1*) and is thought to act in a more global fashion.

Using the framework we propose here, we can investigate the long range, systemic effects of mechanotransduction by building on what has already been reported about the wound healing process, including the presence of peripheral markers of EMT (*15*).

To explore the long range effects of acupuncture, multiomic analysis of the molecular events that occur both proximally and distally from the stimulation point (acupoint) to the target organ, as well as systemically (i.e., in the blood and gastrointestinal (GI) microbiome (*16*)), can be used to construct a spatial analysis. This information can then be enriched with the temporal onset of early gene expression, in addition to later time points (Figure 1A) to construct a systems biology view of the network.

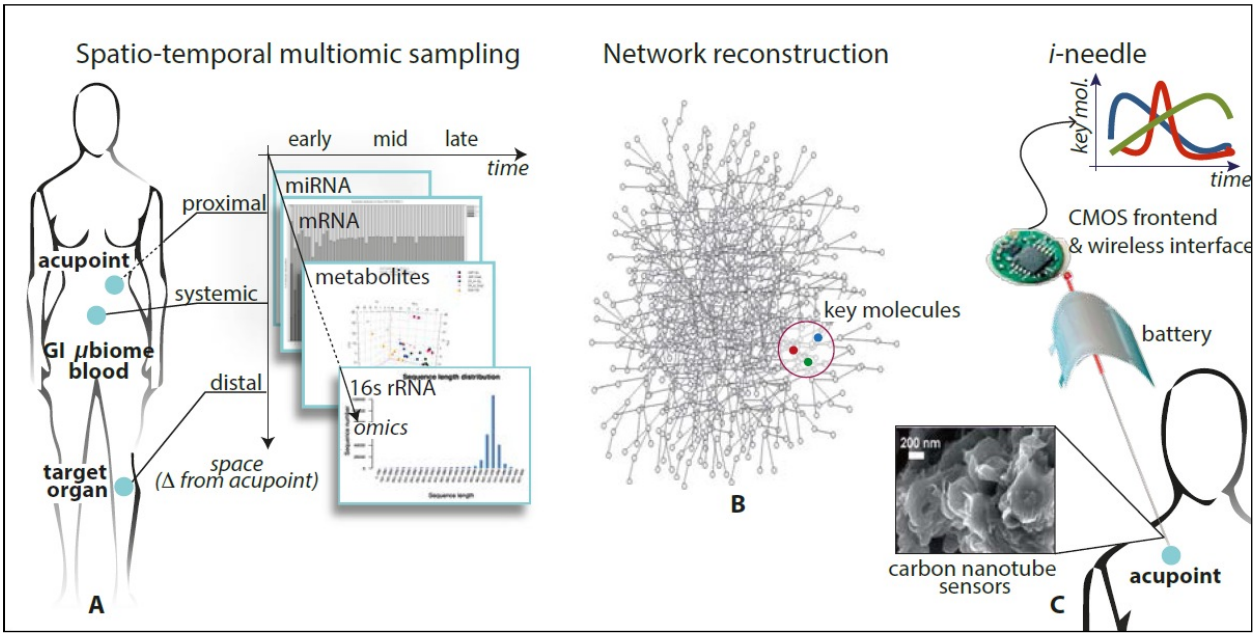

Figure 1. Elements of the *i*-Needle. (**A**) A variety of 'omics techniques can be used to monitor molecular progress across body sites and over time. The pictured model shows the spatiotemporal multi-'omic sampling of the molecular flow of events over the therapy's delivery, from early molecular activation at the acupoint to the peripheral bloodstream and gastrointestinal (GI) microbiome (μbiome) to ultimately reach the target organ. (**B**) Multi-'omic systems biology enables the identification of a network of events, allowing interpretation of acupuncture in terms of a biochemical signaling flow that alters the whole system (body/patient). Network analysis and simulations allow identification of molecules that can be monitored as markers of the progress of the therapy (*17*). (**C**) Diagram showing carbon nanotube (CNT)-based sensor integration to continuously monitor the therapy-induced biochemical progression. Sensors are mounted on an energy-autonomous device that is able to transmit information remotely and in real-time.

In order to build such networks, computational analysis can bring together the different 'omics approaches (Figure 1B) with the temporal and spatial resolution needed to identify new targets for diagnosis and therapy (*18*). This type of network approach can highlight which molecules are important by distilling the relevant ones out of the thousands to tens-of-thousands of interactions among hundreds-to-thousands of molecules, thus also accounting for disparate concauses of a disease along with the proximal (spatially, temporally, or biochemically) pathologies.

Furthermore, the identification of additional markers is made possible with a complementary approach to the high throughput and low sensitivity of these 'omics analyses. This can be imagined in the form of a nanobiochip that is the size and shape of an acupuncture needle (hence, an "intelligent" needle or *i*-needle) (Figure 1C).

Toward this end, we recently created a proof-of-principle miniaturized platform, integrating revolutionary carbon nanotubes and nanographite petals, which can monitor five endogenous human metabolites using highly sensitive and selective nanobiosensors (*19*). The electronics needed to acquire and transfer the detected signal have already been sufficiently miniaturized (*20*) and can be powered by ultrathin polymer-

based batteries (*21*) currently available on the market and able to meet the energy demands of the proposed *i*-needle (~80-130 μAh).

The challenge for the realization of the *i*-needle has already moved from the miniaturization to the integration step (*22*). Progress has already been made, based on recent reports of the measurement and transmission of temperature, pH, and endogenous metabolite data using single-platform enzyme-carbon nanotube hybrid sensors (*23, 24*) .

Conclusions

Overall, it is our hope that this research can provide a more unified approach to understanding the complex nature of patient responses to acupuncture—including effects as diverse as the control of pain, degeneration, and inflammation—and to addressing fundamental issues in acupuncture treatment, such as the frequency of delivery, developing more precise therapeutic indications, and establishing proper "dosage" guidelines. These steps will undoubtedly encourage acceptance of acupuncture as a complementary and/or alternative personalized treatment, with important application in a wide variety of areas including pain control, and degenerative and chronic inflammatory diseases, among others.

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