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Plausible Biomedical Consequences of Acupuncture Applied at Sites Characteristic of Acupoints in the Connective-Tissue-Interstitial-Fluid System

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Acupuncture as one major branch of Traditional Chinese Medicine (TCM)

Traditional Chinese Medicine (TCM) has been in existence for several thousand years, based on the general philosophy that the physiological states of a healthy body are kept balanced. Pathogenesis occurs when such a balance is upset. There have been more and more studies using modern biomedical techniques and animal models on the clinical benefits of herbal TCM in the recent two decades. Fruitful developments have been made on the analysis of the biochemical ingredients of the herbs considered. However, standardization of ingredients of the herbs, the concept of “complex recipe” and a modern theory of herbal TCM described in terms of modern western medicine conception, are still issues of discussion while herbal TCM progresses.

Acupuncture is a part of TCM and the theory of acupuncture mechanism was put forth initially based on a series of hypotheses, conjectures in view of the numerous clinical examples accumulated. Using modern biomedical knowledge to understand the TCM meridians, to practice acupuncture, and to study the benefits of acupuncture seem to be somewhat more readily accepted (compared to herbal TCM) in the west [1]. Zhou et al [2] found 761 English articles published in the year 2006 directly related to acupuncture from various countries, 208 Chinese articles according to Medline search. There are also many other articles and books written in Chinese language published in institutional journals. Thus, English and Chinese are the two main languages for articles and books related to acupuncture research in number.

1.2. The types of acupuncture research carried out internationally

1.2.1. *Clinical acupuncture research*

Clinical data/reports have been collected with a long history in China on the benefits of treating various disorders of health, using the TCM concept of “energy flow” along the meridians, and certain basic principles stated in classics (without direct verification). Some such books incorporating new concepts in biomedical science also appear in the recent two decades or so. These writings have been published mainly in the Chinese language [3-4]. There are also clinically oriented books written by acupuncturists in acupuncture centers/institutes of western countries, Japan and Korea. Those written in English are focused more on using various modalities of acupuncture as means to treat pains [5-6]. Some of these writings use the concept of triggered points rather than TCM acupoints, and the benefits of acupuncture are usually associated entirely with signals transmitted through the nervous systems [7].

1.2.2. *Basic evidence-based acupuncture research*

The fast development of biomedical science with new techniques and concept has helped to inject enthusiasm and good attempts/results from scientists globally using animal models, in vitro experimentations, or clinical settings to analyze the following general issues: (I) the anatomical characteristics of the meridian channels/acupoints; (II) to investigate on how the acupuncture signals could be transmitted from the acupoint to other parts of the body, and via the meridian channels; (III) the plausible physiological consequences of acupuncture in action; these consequences include both biophysical and biochemical aspects.

1.2.3. *Accumulation of a huge data base from biomedical science/engineering research which is indirectly related to the mechanism of acupuncture in action*

For a long time, biochemical force is considered to be the most fundamental force in cell signaling involved in physiological processes, and in initiating cellular response to external stimuli. However, research during the last 20 years on cell signaling and cellular structure reveals that mechanical sensors (integrins with the focal adhesion complexes and mediators) exist at the cell membrane to bridge the extra-cellular matrix (ECM) and cytoskeletal elements in transmitting mechanotransduction signals. Then cytoskeletal elements build up prestress and contractile structures, joining the nucleus to the cytoskeletal base of the mechanical sensor unit. Mechanical stimulation initiates a cascade of cytoskeletal remodeling. Thus, the cell nucleus, via mechanotransduction, can react directly to a wide range of mechanical stresses. It has also been found that mechanotransduction, which exists between adjacent cells, and between extracellular fluid and cells, plays even more fundamental roles than biochemical interactions [8-11].

In fact, mechanotransduction is a key fundamental concept to physiology, ensuing the organism's structural stability and development. A lot of new experimental data, new conceptual knowledge, plus consequential deductions related to mechanotransduction have been

published based on modern knowledge of biophysics and biochemistry. These results provide important clues to the understanding of the basic acupuncture mechanism.

1.3. Objectives of this chapter and the settings in presentation

Based on the remarks mentioned in Sections 1.2 (B and C), I would review first briefly in Section 2 the basic knowledge of mechanotransduction via solid and fluid objects for convenience of more in-depth discussion about plausible consequences of acupuncture in later sections of this chapter. Evidence and reasons on the occurrence of the meridians/acupoints in the connective-tissue- interstitial fluid (CTIF) system is presented in Section 3; the typical anatomical features of acupoints are briefly highlighted. Section 4 starts with a description on the initial response of the CTIF system when manual acupuncture is applied to a typical acupoint in the CTIF system. Section 5 is devoted to a discussion on the plausible consequence of acupuncture when the mechanical stimulus reaches the ECM of cells building the blood vessels, with support from results of some clinical studies and in vivo animal investigation. Note that an acupoint is found to be near nerve endings and nerves. Various types of neural signals are found to be triggered with evidence from laboratory and clinical studies. Therefore a brief analysis on the neural aspects of mechanotransduction is carried out in Section 6. In Section 7, the evidence on the existence of fluid flow along channels (which are not blood flows) correlating to the TCM meridian channels is presented. The importance of maintaining a proper flow of the interstitial fluid to health is emphasized. The issue on whether acupuncture could help to keep the homeostasis of such fluid flow in the body is discussed in Section 7. Section 8 is catered for a brief discussion on the degranulation of mast cells, which have been found to be near typical acupoints. The plausible release and activation of transforming-growth-factor- β (TGF β) upon unfolding of an abundant protein, the fibronectin in the ECM of fibroblasts is discussed. Since TGF β activates local stem cells specification, and mast cells sustain fibroblasts, the release and activation of TGF β and mast cell degranulation are discussed together as part of the function group for immunity and wound repair processes in Section 8. The plausible consequences of acupuncture described in Sections 4-8 is summarized with the help of a flow chart in Section 9. This section also presents some frontier issues and new ideas for future acupuncture research. Section 10 concludes this chapter. Unless otherwise stated, acupuncture refers to manual acupuncture, as this chapter is focused on the consequences of acupuncture arising from mechanotransduction.

2. Basics of mechanotransduction, relevant to discussion in this chapter

2.1. Tensegrity of living organism on earth and mechanotransduction

Any living organism living on the massive earth is subjected (a) to the gravitational force which manifests as a mechanical pull on every part of the body continuously, and (b) frequently to external mechanical and other forms of stimuli from other objects.

Research during the past two decades on cell signaling and cellular structure indicates that (1) external stimuli of mechanical or electrical nature control many fundamental biochemical interactions; (2) tissues are composed of a network of “connected” cells and proteins, rather than individual cells plus proteins in a body fluid. Putting these two observations and recalling the two “disturbances” (a) and (b) on organisms on earth just stated above, one is inclined to consider that a special architecture system must be at work in each living entity particularly suitable for transmission of mechanical signals, if it is to remain as that entity. Such an architecture system is one important basis for allowing acupuncture signals to be transmitted to certain parts of the body. Ingber and other workers have realized the importance of this living architecture and developed the tensegrity model of a cell [12].

Mechanical signals are inferred to be transmitted through mechanotransduction in principle to various parts of the body based on the hierarchy (systems within systems) nature of the body, with connective tissues (CT) embedding other tissues/organelles/organs. The mechanical forces involved in mechanotransduction through the body are mainly tensile and compression for solids, and shear stress (which is the friction force of fluid flow acting on the surface of cells) and pressure for fluids [13].

Note that mechanosensitive ion channels, which are also “fundamental components” for processes of mechanotransduction, were discovered long ago (1950) among neurons [14]. Mechanosensitive channels were documented in various other cells in the 1980s [15]. During that time, the tensegrity concept was not brought in from architecture.

2.2. Mechanical signals at the cell membrane can cascade a series of signals reaching the cell nucleus – Integrin starts the story

Integrins, being composed of alpha and beta subunits with various combinations, are cell membrane receptors which can bind to proteins in the ECM with specificity [16]. There is ample evidence that the basic mechanism of ECM- cytoskeletal elements connection is mediated by integrin clustering [8, 11]. When the integrins are stimulated mechanically, they connected intracellular proteins like focal adhesion kinase (FAK), Src-family kinases. Activation of these kinases triggers off a series of pathways associated with cytoskeletal remodeling. In particular, it was demonstrated that the stress-induced mechanical strain stimulated conformational activation of α – and β - integrins of NIH3T3 cells [17]. Such activation was mediated by phosphoinositol 3-kinase and was followed by new integrin ligand binding to ECM proteins, which then mediated c-Jun NH2-terminal kinase (JNK), initiating a series of cellular responses without upsetting the existing integrin-ECM binding. In general, responding to mechanical stimulation, there is growth of focal adhesion, leading to a stress-dependent increase in cytoskeletal (CSK) stiffness. The CSK stiffness changes because there is rearrangement of the microfilaments (MF), microtubules (MT) and intermediate filaments (IF). Since these structures join the nucleus surface after such CSK remodeling [11], expressions of different genes are affected to produce the relevant proteins to maintain the cell integrity and initiate the necessary physiological processes [18].

2.3. Intercellular communication through mechanotransduction – Connexin plays one key role

Various connexin proteins build up gap junction proteins between adjacent cells [19]. It has already been known, based on analysis of synchronous contraction of myocardial cells, that the gap junction proteins participate in the transfer of mechanical stimulation, indicating that mechanotransduction can be “passed on” between adjacent cells of a tissue/organ [20]. In the same work, it was shown that electrical synaptic transmission between nerve cells depended also on the integrity of gap junctions. Instead of building a passive gateway for the transportation of electric charge and relatively small molecules, the connexin protein Cx43 has been found to bind directly or indirectly to some intracellular proteins like caveolin, 20- β -catenin, tubulin, src which have various functions, supporting the idea that gap junctions take part also in mechanotransduction [21]. The correlation between activation of connexin and acupoints will be followed up in Section 9.

2.4. Intra and inter-cellular calcium ions oscillations are involved in the mechanotransduction processes of many cell types, including fibroblast

Many biochemical pathways involving different secondary messengers occur consequentially to a simple mechanical stimulus at a cellular mechanical sensor [22, 23]. First, there is ample evidence that intra-cellular Ca^{2+} oscillations and inter-cellular Ca^{2+} waves are involved in the mechanotransduction processes of many cell types, including fibroblasts, though the term mechanotransduction might not formally be put in all the articles at that time [24-27].

Recent studies also identify changes in cellular Ca^{2+} concentration to be one major step of translating mechanical forces and deformation at the cell membrane into biochemical signals [28]. Note that Inositol 1 4, 5-Trisphosphate (IP3) - dependent or independent pathway may be involved in these Ca^{2+} activities in various cells [29, 30].

The question of how intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ oscillations could affect mitochondrial functions has been investigated. It was demonstrated in [31] that IP3-dependent intracellular $[\text{Ca}^{2+}]_i$ oscillations were transmitted to the mitochondria of hepatocytes, based on the fact that Ca^{2+} regulates some calcium-sensitive mitochondrial dehydrogenases (CSMDHs). This finding suggests that the mitochondria are tuned to oscillating $[\text{Ca}^{2+}]_i$ signals and different ranges of frequency are related to different potential activities of CSMDHs. As the function of the mitochondria is a very important aspect of cells and tissues, the expected consequence is a cascade of physiological cellular events. On the other hand, the characteristics of these intra-cellular and inter-cellular calcium activities are dependent on the amount of extracellular calcium ions (in the “extracellular calcium store”) associated with the stimulated cells [32]. Moreover, variation in cytoplasmic Ca^{2+} activities was found to correlate with those of nuclear calcium. In fact, it was demonstrated that cytoplasmic calcium and nuclear calcium activities control gene expressions in different ways [33, 34]

In vitro experiment [27] using 3T3 fibroblasts demonstrated that stretching forces applied through the flexible substrates (mimicking the CT) would induce increase in intracellular Ca^{2+} concentration due to influx from extracellular calcium store [27]. These fibroblasts

(then) showed cytoskeletal contractility, an aspect crucial to transmission of acupuncture signal through the CTIF system. The correlation between calcium concentration and acupoints will be discussed in Section 9.

3. The CTIF system as platform for acupuncture signals to cause biomedical effects via mechanotransductions of various kinds

3.1. Crucial characteristics of the CTIF system relevant to providing means for transmission of acupuncture signals

Connective tissue (CT) is the most abundant tissue of the body. The components in CT are the collagen fibers of various types [35], glycoproteins like fibronectin, laminin, tenascin, elastin and various subclasses of proteoglycans. Among these sub-classes, hyaluronan is the dominant glycosaminoglycan serving as the backbone for the assembly of other glycosaminoglycans in the CT. These proteins form different networks (with different combination of components) in the extra-cellular matrix (ECM) associated with different tissues/organs “wrapped” by the CT [36, 37].

The interstitial fluid serves to transport nutrient proteins, ions, oxygen and water from the capillaries to the general tissues for their functions. It also removes cellular excreta, unused proteins to the lymphatic system as well as transports CO₂ from the tissues to the venous capillaries. The proteoglycans of CT form extremely thin fibrils which interact with the surrounding interstitial fluid (IF) to form a gel-like ground substance [38, 39], allowing slow flow for proper distribution of nutrients and processing of excreta.

The connective tissue interstitial fluid (CTIF) system forms a body frame with connected layers which embeds the neurovascular tracts, connects tunicae around the visceral organs, and extends to form the periosteum, nourishing the bones [38-41]. Nerve fibers are densely populated around acupoint sites in the CTIF system. These nerve fibers innervate blood vessels, lymphatic vessels, with different types of endings serving as specific or polymodal nociceptive receptors [42]. The residence cells in the CTIF system are lymphocytes, macrophages, adipocytes, plasma cells, eosinophils, fibroblasts, chondroblasts, osteoblasts, stem cells and mast cells [43]. Fibroblasts sustain collagen fibers [44] while mast cells upkeep the proliferation of fibroblasts [45]. The “organ/adherent cells” are defined here to be those forming part of the “non-CTIF” tissue/organ like blood/lymphatic vessel cells, nerve cells, visceral organ cells.

Without reference to special particulars of the acupuncture mechanism, some earlier studies already indicated that fibroblasts throughout the connective tissues system were connected among themselves [46, 47]. It was found that fibroblasts in different tissues grown in cell culture were seen to contact one another with gap junctions at contact points [48-50]. Moreover, many cryptic binding sites of the fibronectin modules have been identified; these binding sites are hidden when the fibronectin proteins are in the folded state. Unfolding the fibronectin by mechanotransduction through the CTIF system could unfold the modules in-

to fibrils, releasing some of the already bound growth factors as well as exposing other binding sites to take part in biochemical activities; see review of [51].

3.2. Early evidence of the involvement of connective tissues (CT) during acupuncture in action, up to year 2000

According to the TCM classics [52a], which was written a few thousand years ago, it was hypothesized, based on clinical experience using acupuncture, that “..... the twelve meridians go through regions between muscles, are deep and cannot be seen.....”. Now we know that in between the muscle spindles are connective tissues with deposited proteins, as part of the CTIF system. So the correlation of the connective tissues with the meridians was already hinted/stated in ancient Chinese classics. A series of research work starting from 1980s leads to the analysis on the anatomical structure of the acupoint sites (of cadavers) and it was found that acupoints of the “earth positions” [52b, 52c] were three-dimensional spaces being close to nerve endings or branches of nerves, blood vessels and lymphatic vessels; these regions or “spaces” referred to always include also one type of connective tissues or another within the CTIF system [53-55]. Direct verification that acupoints are actually within the CTIF system was not directly reported until science and technology developed to that stage around 1990s.

In 1992, it was reported in [56] that after sparrow-pecking and twisting manipulation of acupuncture needles to acupoints BL23, BL24, BL25 of human subjects until de qi was felt, some transparent materials were found to be attached to the needles by microscopic analysis. The materials contained collagen fibers, elastic fibers, fibroblasts, mast cells, macrophages and adipocytes. In another study [57], based on morphological analysis of five cadavers, the 3D structure of the acupoint SP6 was found to contain nervous, vasculature structures embedded in connective tissues. Dang et al in [58] used three cadavers and inserted needles to 11 acupoint sites of the Lung Meridian. In doing so, the acupuncturist also cast the needle to each acupoint on a healthy male volunteer until de qi, and carried out computerized tomography x-ray examination of the needle position, to be compared with that exercised on the cadavers. Nine out of the eleven acupoints of the Lung Meridian were found to be on the periosteum layers of the related bones. Using three cadavers, the investigators [59] carried out an anatomical analysis of all the acupoints of the GB meridian channel below the head and found that from GB20 to GB44, 18 acupoints were mainly associated with the periosteum and interosseous membrane, the other 7 were related to fascia, epineurium of the nerve and articular capsule of the knee. All these anatomical structures are in fact connective tissues of one form or another. Fei et al employed a video microscope [60] to examine the specimens around the acupoint ST36 of cadavers and discovered that the anatomic structure of capillaries, nerve plexus and lymphatic ducts formed a complex structure with connective tissues as basis. They also analyzed the anatomy structure of 73 acupoints along three different meridians of three cadavers and reported that all these acupoint sites were associated with connective tissues. Other similar characteristics have been reported along the acupoints of the Stomach Meridian [61]. So many acupoints were found to be within layers of connective tissues in, at least, all these studies.

3.3. Evidence of the involvement of connective tissues during acupuncture in action, starting from the twenty-first century

To probe the mechanism of acupuncture signal transmission, Langevin's group produced a series of works, demonstrating that connective tissues of rat abdominal wall explants would wind around a rotating needle but not around an inserted one [62]. The suction force and torque experienced by an acupuncturing needle in vivo during de qi were measured quantitatively by the machine they built. It was demonstrated that fibroblasts in the stated explants would change the morphology from the "quiescent" round shape to elongated one when the collagen fibers whirled around the (acupuncture) needle. In 2003, Ding et al [63] repeated the above-stated force and torque measurement with a simpler machine. Comparing fig.6 of [62] and fig.8b of [63], it appears that fig.8b gives an additional feature ("vibrating-like" wave, representing relaxation) to verify the involvement of elastic anatomical tissues during the relaxation part of one complete action of winding and unwinding in the in vivo experiments of subjects.

Later, more than 80 % of acupoints were found to coincide with intermuscular or intra-muscular connective tissue planes of postmortem tissue sections of the arm [64]. Images were obtained from a digital data set derivable from 8556 slices of a female cadaver [65]. Likewise, similar images were obtained from 9232 slices of a male cadaver [66]. Based on such datasets, 3D images were obtained to generate the frames of the connective tissues for the whole female and male bodies [41]. They located the positions of acupoints according to TCM on such frames and reported that for one body, 361 acupoints were all located in various types of connective tissues, with a maximum of 204 in the intermuscular (loose) septa.

Further, it was demonstrated in [67] that needle-rotation-induced morphological change in mouse fibroblasts explants was inhibited by inhibitors of actomyosin activity, Rho kinase and Rac signaling. Analyzing these intracellular biochemical pathways, that "tension-induced signaling by acupuncture would lead to cytoskeletal remodeling of fibroblasts" was considered to be directly verified in the explants experiments. These changes in cell shape required the presence of both intact microtubules and microfilaments, implying an active, cytoskeletal-dependent mechanism.

4. Response of the CTIF system to acupuncture at a typical acupoint in the loose connective tissue layer

4.1. Fibroblasts in their quiescent state are not isolated, intact cells; traction of fibroblast can trigger rapid global self-organization of the collagen matrix system

It was shown that slow local motion of collagen fibers driven by fibroblasts induced rapid global self-organization of collagen gel (in minutes) in in vitro experiments [68]. Later, another in vitro study showed that fibroblasts in their quiescent state formed a whole body-like network, with their processes in close proximities [69], implying that fibroblasts are not really "isolated" cells. It has been demonstrated by another group that fibroblasts interact

with 3D relaxed collagen matrices to form dendritic type of extensions without the alignment of cytoskeletal microtubules [70]. However, at a high tension state (typical of cells on “coverslips” in in vitro settings of such types of experiments, or consequence of acupuncture rotation in the loose connective tissue), cells spread with lamellipodial extensions, requiring the function of microtubules, meaning that the fibroblasts are exerting attraction force (back) to the ECM matrix if the mechanical stimulus is strong enough. It has also been found further that migration of fibroblasts can remodel the architecture of the collagen matrix both locally and globally [71], substantiating and adding more to [68]. All these studies demonstrate that there is a strong mechanical coupling between the fibroblasts and elements of the connective tissues system, as a basis of transmitting mechanical signals through the CTIF system, particularly when the tension of the collagen fibers reach a certain magnitude.

4.2. The rapid global self-organization of the collagen matrix system is initiated by fibronectin fibrils formation

Despite the experimental discovery of the rapid self-organization of the collagen matrix as a phenomenon, stated in Section 4.1, a scientific link is still missing as to understand the basic mechanism on how such organization could occur rapidly to distances much greater than cell size. Note that a number of proteins are attached to the ECM of cells with specifications (Section 3.1). Among them, fibronectin (Fn) is produced by most cell types into their ECM [72]. Using fluorescence resonance energy transfer techniques, it has been found only several years ago in [73] that traction force of cells could stretch an initially nodular assembly of Fn (with quaternary structure) to many times its initial length. It is noted that the unfolding of Fn played an import role to outside-in and inside-out mechanotransduction due to cell traction or when mechanical stimulus arrives the membranes of cells. Thus, the in vitro experiments suggests that in vivo acupuncture in action causes not only morphological changes of fibroblasts, but also (as a consequence) an anatomical change-unfolding of Fn, leading to formation of Fn fibrils in the collagen matrix. The CTIF system is thus mechanically well connected for efficient mechanotransduction. Whether other proteins like laminin or tenascin (Section 3.1) would participate in a different or similar way to assist mechanotransduction is still a future research project. Now we understand at least Fn plays one key role in global self-organization by forming fibrils.

4.3. Inter- and intra-muscular connective tissue cleavage planes favor mechanotransduction to longer distance – suggestive of high efficiency of mechanotransduction through the meridian channels

A high correlation between inter- and intramuscular connective tissue cleavage planes and acupoints (around 80 %) and meridians (around 50 %) was found via analysis of cadaver sections [64]. Though the exact percentages different in other studies [41, 58, 60], by far the majority acupoints were found in the loose and other types of connective tissues, including that building up the periostia to join the bones. To set up in vitro models of the intermuscular plane, type 1 collagen was cast in circular gels of different radii, elliptical gels with quantified major and minor axes, matching the large and small circu-

lar gels respectively [74]. Collagen was also cast in planar gels with constraint on two sides. Acupuncture needles were inserted into the gel specimens and rotated by motors, mimicking acupuncture in action. It was found that rotation in planar gels with constraint on two sides, elliptical gels, circular gels generated the strongest, less strong and weakest alignment of collagen fibers respectively. With fibroblasts in the gel specimens, the phenomenon of durotaxis was demonstrated [39]. In other words, fibroblasts followed alignment of collagen fibers, with denser population along stronger alignment. Result of such in vitro experiment, in view of the special anatomical characteristics of acupoint sites, supports the notion that efficiency of mechanotransduction through the acupoints and meridian channels is relatively high, compared to non-acupoints, when induced by acupuncture action.

5. On the issue of peripheral blood vessel dilation resulting from acupuncture

5.1. Recent revisit to the long standing question – Why would muscle compression on blood vessels trigger immediate vessel dilation?

Biomedical scientists have been trying for years to explain the daily-experienced occurrence of the immediate increase in blood flow to skeletal muscles at the onset of exercise.

In 2006, rat soleus arteries were isolated and pressure compressions were applied in a series to the vessels [75]. It was found that dilation of these arteries was elicited by brief periods of compression mimicking extravascular contractions, substantiating the stated daily experience at the onset of exercise. Scientists still have to find out more about the mechanism behind the phenomenon of “self-regulatory type” of dilation.

5.2. Recent discovery: Unfolding of fibronectin fibrils in the ECM of vessel cells as one key clue for Nitric Oxide-mediated local dilation

Using intravital microscopy in in vivo animal experiment, it was reported for the first time [76] that fibronectin (Fn) fibrils in the CT near blood vessel would transduce mechanical signals from contracting skeletal muscle to trigger a fast, specific and reversible local vessel dilation mediated by nitric oxide, the commonest vessel dilator. The active part of the Fn fibrils was found to be type III-I of Fn. Moreover, Fn, administered intravenously, was found to assemble quickly into elongated, branching fibrils in the extracellular matrix of intact cremaster muscle, showing active polymerization of Fn in areas adjacent to blood vessels. These two sets of experiments indicated that unfolding of the fibronectin fibrils of adult connective tissue plays a dynamic role in regulating both vascular responses and vascular tone. Though the experiment was meant to find out why exercise caused vessel dilation, the important result of these studies is that mechanotransduction, when reaching the ECM of vessels could cause unfolding of Fn, triggering

biochemical events leading to nitric oxide (NO)-mediated vasodilation (various aspects of NO generation are discussed also in Section 9.4). This series of studies put forth the notion that blood vessels have self-regulating system to tone the dilation in response to external mechanical disturbance, for survival of the organism.

5.3. Direct evidence of acupuncture-induced local vessel dilation

In a clinical setting, the levels of NO and the protein Interleukin-2 (IL-2) contents of peripheral blood were reported to be significantly higher in 42 subjects after warm needling at ST36 [77]. Using animal models, it was demonstrated [78] that electro-acupuncture at ST36 reduced blood pressure by activation of two nitric oxide Synthases (eNOS and nNOS, Section 9.4). In another clinical study with 20 subjects, it was demonstrated that acupuncture would cause enhanced release of NO locally, leading to improved blood circulation. In particular, NO concentration in the plasma from the acupunctured arm was significantly higher after acupuncture. Blood flow in the palmar subcutaneous tissue of the acupunctured arm was also increased [79]. Moreover, acupuncture was performed at LU7 of 56 subjects [80], and the skin blood flow was found to be raised along the track from LU7 to LU5, using a laser Doppler flowmeter.

5.4. The observation of infrared radiant tracks along the TCM meridians on the skin during acupuncture and the recent discovery of adenosine triphosphate (ATP) enhancement at acupoint region after acupuncture

A number of studies have been carried out to measure the temperature of different parts of the skin (particularly the limbs) after acupuncture was applied to a diversity of acupoints of human subjects and animals, using thermograms with resolution about 0.1 degree Celcius [4, 81, 82]. Infrared radiant tracks were detected along lines correlating with various TCM meridian channels on the skin during acupuncture. Later, specimens along and away from outside of these infrared radiant tracks of animal models were taken and it was found that both the P substance and ATPase were higher with statistical significance along the meridian lines [83]. In another animal study [84], points along the Du meridian were taken as test points and points 2.5 cm sideway to the test points were taken as reference. The oxygen partial pressure PO_2 values in the deep tissues along these test points were found to be significantly higher than that of the reference points. The above results suggest that higher metabolism rate was the source of the infrared radiant tracts observed on the skin, subsequent to acupuncture action.

Recently, it was found in a mice model that acupuncture at ST 36 triggers an increase in the ECM concentrations of ATP, ADP, AMP and adenosine. Abundance of these basic units of bio-energy indicates occurrence of high metabolism rate [85]. The appearance of infrared radiant tracks on the skin part of the meridians can be interpreted as enhancement of blood flow through the blood vessels (arising from activation of eNOS or non-NOS enzymes which cause vessel dilation) along the meridian channels during/after acupuncture, leading to a higher metabolic rate.

6. Transmission of the acupuncture signals through mechanotransduction from connective tissues to the nervous system

6.1. Acupoints are close to various types of nerve receptors

It has been hypothesized/inferred with evidence as stated in [39, 60, 86, 87] that acupoints are close to high density sites of polymodal and specific nociceptive receptors, neurovascular structure, lymphatic vessels, and mast cells. In fact, high density of nerve endings was also found at many acupoint sites of rat models recently [88]. All peripheral nerve fibers are embedded by various CT layers (endoneurium, perineurium, epineurium) with epineurium forming the outermost layer. Thus acupuncture applied at the acupoint sites which are embedded in the CTIF system would send mechanical signals readily to the mechanical nerve sensors close to the acupoints. Upon receiving mechanical stimulation, the mechanical receptors of nerve cells would have some of the cations and Ca^{2+} activated [89]. The in-flux and out-flux of the associated ions leads to the appearance of the action potentials which propagate as signals through the nerve fibers. Evidence-based result suggests that mechanotransduction originated from acupuncture could send signals through the skin, as well as through reflexes carried out by nerves deeper down, to be briefly specified below.

6.2. Acupuncture could initiate signals via mechanotransduction to travel along the skin level of the meridian channels based on animal model studies

In a series of study, 125-I-tyrosine was injected to animal models [90-92]. The epidermis and dermis part of the skin was exposed to x-ray films for weeks. Longitudinal distributed lines with rich sympathetic substance (neurotransmitters) were found on the skin. These lines were inferred to be correlated to the skin parts of the known TCM meridian channels. Acupuncture at ST36 was demonstrated to relief pain of the rabbit model, but injection of inhibitors of neurotransmitters or cutting the part of the skin en route the Stomach Meridian at the back of the animal model abolished the analgesic effects. These studies provide evidence that acupuncture could cause signals to be transmitted along meridian channels at the skin level. The signals propagate as if jumping over nerve endings. Other independent studies [93] documented that evoked potential could be transmitted across skin nerve endings of the Urinary Bladder Meridian of the bullfrog model using electrophysiology technique. So far neural transmission was interpreted as axon reflex along the skin employing hairy animal models in the above four references. More detailed analysis is needed to make more concrete statement on the stated skin-phenomenon associated with the application of acupuncture to “non-hairy animal models”.

6.3. Acupuncture leads to “external-internal connection” (a TCM concept) by way of somato-visceral organ reflexes based on animal models studies

In Chinese medicine the external-internal connection has been emphasized for several thousand years [52c]. In modern biomedical science language, the function of visceral organs is regulated by the autonomic nervous system and the function of the skin and skeletal muscle

is regulated by the somatic nervous system. Since specific muscles are connected to a visceral organ/system in general, the somatic nervous system also participates in the regulation of the visceral organ. Thus the external (acupuncture at sites near nerve sensors) and internal (visceral organs function) connection via the somato-visceral organ reflexes are explainable physiological pathways [94-96]. As the somato- and visceral-nerve fibers are connected to one or more spinal cord segments, and eventually to the brain, the organization centers of the somato-visceral organ reflexes can be at the spinal cord segment(s) or the brain [94]. As noted in [96], the somato-visceral reflexes can be mediated through four major pathways: (i) the axon reflex which has no direct automatic involvement but performs as if there is automatic activation; (ii) the spinal reflex characteristics of segmental organization; (iii) the medullary reflex which is integrative in nature; (iv) supra medullary reflex which is related to hormonal secretion like sweating and regulation of cerebral blood flow.

6.4. Acupuncture induces immunity-mediated or neurogenic inflammation around the region of stimulus

Some somato-visceral neurons, having their cell bodies in the dorsal root ganglia, behave as afferents as well as efferents by releasing neuropeptides from their peripheral terminals. These peptides induce a cascade of changes, collectively called “neurogenic inflammation”, specified by two general events [97]: (i) Antidromic vasodilation (ADV), the increased blood flow seen in tissues on stimulation of the sensory dorsal roots. (ii) Plasma extravasation.

The C and A delta neuron endings with abundant nociceptive receptors are hypothesized with evidence to be acupoint sites [39, 60, 86, 87], and they are being involved in such inflammation. Antidromic vasodilation (ADV) is only clearly seen when C-fibers or some of A δ fibers are excited [97, 98]. What our body system tries to do is “reasonable” and obvious microvessels (particularly the capillaries) dilate, and increase the permeabilities of the vessel walls so that blood flow is increased together with enhanced exudation of plasma to the injured site where acupuncture was applied. During this time more leukocytes, which are activated by the hormones, are released from the nociceptive sensory receptors. These leukocytes can migrate from the blood to the interstitial site, and together with the “residence” immune cells of the CTIF (macrophages, eosinophil,), they carry out their basic immunity duties, while the excreta are being carried away by relatively larger fluid volume to the lymphatic vessels to be processed at the lymph nodes. That acupuncture would lead to neurogenic inflammation has already been analyzed in [87]. Though acupuncture-induced neurogenic inflammation could bring benefits (details like pain treatment are outside the scope of in this chapter), the effect must be regulated/fine-tuned. For example, manual acupuncture has been documented to modulate capsaicin-induced edema, a phenomenon within the general concept of neurogenic inflammation [99, 100]. Concerning the fine-tuning aspect, there is evidence that the nervous system can regulate the inflammatory response reflexively in real time [101]. To find out the modality of acupuncture that could regulate neurogenic inflammation by stimulating selective and reversible reflexive neural systems is perhaps another topic of acupuncture research in the future.

6.5. Acupuncture can trigger signals through afferent nerve fibers to reach the brain – MRI demonstrations

Using magnetic resonance imaging (MRI) technique, investigations have been carried out to analyze the parts of the brain being activated in response to acupuncture or electroacupuncture applied at acupoints and non-acupoints. It was found that acupuncture stimulation of acupoints has specific effects on cortical neuronal activity, but not so for non-acupoints [102]. The integrated response of the human cerebellar system to acupuncture stimulation at ST36 was found to be correlated with the psycho-physical response [103]. There was evidence suggesting that chronic pain patients responded to acupuncture differently than healthy normal subjects, through a coordinated network including hypothalamus and amygdala [104]. However, a recent study points out that nonferromagnetic needles need to be used during acupuncture if MRI measurement is to be carried out [105]. I would note that the magnetic field inside a MRI tube is typically ~ 15, 000 gauss, whereas the earth's magnetic field is around 0.5 gauss. The magnetic field at the point of acupuncture application has to be well-shielded during MRI scanning, if the needle is ferromagnetic, for obvious scientific reasons. Therefore, I could not comment on the MRI results unless proper needles are used in this type of studies or proper magnetic-shielding is clearly specified in the report.

Another alternative is to employ real time source imaging analysis using multi-channel electroencephalogram (EEG) to analyze the brain function [106] during acupuncture in action. It might be possible to make more concrete statement on how the brain responds in real time to the action of acupuncture employing this new neural imaging technique using dipole source analysis in the future.

6.6. Mechanotransduction induces release of vasodilatory neuropeptides from nerve endings, a very recent study

Acupuncture was applied to acupoint Hegu (L14) of subjects, while the frequency content of skin blood flow signals was measured using Laser Doppler flowmetry (LDF) simultaneously [107]. It was demonstrated that needling L14 significantly increased blood perfusion at the acupoint, but not at nearby non-acupoint sites. Based on a wavelet transform analysis technique of the LDF data [108], it was inferred that the resultant blood flow increase was caused by the release of vasodilatory neuropeptides from nerve endings, which subsequently interact with sympathetic vasoconstriction neurons. Thus apart from more direct NO-mediated vessel dilation triggered by mechanical signals applied to vessel cells discussed in the last section, acupuncture could induce vessel dilation through interaction with nerve endings.

7. Acupuncture may influence the flow characteristics of the interstitial fluid, leading to biomedical consequences

7.1. Evidence of the interstitial fluid flows correlating with some TCM meridian channels using radioactive tracers

Starting from 1960, Chinese researchers began to inject radioactive materials in acupoints and recorded the radiation using scintillation counter outside the test body. The first formal

report released to the public appeared in [109]. Tiberiu et al then [110] recorded the radioactive trace to follow from UB61 to UB67, away from the heart [110]. It was found also in [110] that there was a “stationary time interval” during which propagation did not proceed at all and this interval varied among individuals and among meridians, having a mean of 37.28 s. Later, radioactive material was injected into acupoints of the extremities (fingertips and those around the ankles) of 94 volunteers. The longest radioactive track detected was identified to be along the Spleen Meridian, to a length of 85.87 cm and the shortest propagation path was 33.21 cm, correlating to the Large Intestine Meridian [111-113]. These authors concluded that the flows were not along blood vessels.

7.2. Evidence of fluid flow along channels of low dynamical resistance in animal models using pressure transducers

While the existence of the interstitial fluid is well established in physiology, fluid pressure measurement in vivo was progressed very slowly due to experimental difficulty. Guyton [114] and Levick [115] developed two basic methods [116] using mechanical transducers connected to special needles which were inserted to sites of investigation in vivo. Analyzing signals obtained from the transducers give information on the relative pressure of the interstitial fluid. Zhang modified these methods and to measure the fluid pressure and discovered that many sites of relative low fluid dynamic resistance existed in the subcutaneous connective tissue layers of animals (rabbits, pigs) and humans [117, 118]. From such series of studies, he inferred that the meridians were interstitial fluid channels flowing with different speeds at different sites, substantiating the existence of flows correlating with some TCM meridians and general characteristics of “uneven flow rates” discovered in the radioactive tracer experiments.

7.3. Can acupuncture influence the flow characteristics of the interstitial fluid in the CTIF system?

It is well-known in classical physiology that the IF flow characteristics are controlled by the hydrostatic pressure P_{if} of IF, the colloid osmotic pressure (Starling’s law) as well as the lymphatic draining strength. While the vasculature system is well studied, the features of IF flow and variations of P_{if} transports are relatively un-explored. If there is a difference of P_{if} between two adjacent points in the interstitium, IF tends to flow to balance such a pressure difference. Since gravity also acts on the IF, at any instance, the P_{if} in different parts of the interstitium are different, and the values of P_{if} depends on the posture of the body [119]; a recent revisit has been paid to analyze how would the interstitium operate as a dynamic interface for water and solute distribution over the body [120]. As the IF flows through a complicated structure of fibers, proteins, cells in the interstitium, intuition argues that the CT structure would influence IF flow characteristics. If a subject is to remain in the (healthy) physiological state, the IF must have the proper flow speeds at various sites for healthy distribution of nutrients and drainage of excreta (Section 3.1). There is evidence that the integuments of the residing cells and growth factors in the CTIF together play active role tending to keep IF homeostasis [121, 122]. On the other hand, it is documented in [123] that P_{if} in

(breast) tumor is increased, caused by tumor-associated angiogenesis, and the IF flow speed becomes faster. Such rise in P_{if} inhibits pharmaceutical chemicals to reach the target tumor; moreover, cancer cells might be driven to the lymphatic vessels nearby. The aspects on how physical force of the flowing IF would regulate tumor invasion were analyzed in [124]. The issue on the synergies between lymph drainage and flow-induced mechanotransduction in stroma of cancer cells is being studied very recently [125]. Thus controlling proper P_{if} seems to be important for health, but biomedical scientists are still in the “infancy state” of IF research. When we rotate a needle back and forth during acupuncture, in addition to sending mechanical signals along the CTIF system, intuition seems to support the idea that there is a redistribution of tension among the collagen fibers affected. Since the collagen fibers of the CT are embedded in the IF, acupuncture gives room for the IF to redistribute the P_{if} also. Thus I hypothesize that acupuncture serves as a toning process of the IF flow, tending to keep homeostasis of P_{if} along the meridian channels (see a related article [126]). Further research along this line is also important, keeping in mind that P_{if} rise in tumor is the consequence of a pathological process.

8. Acupuncture causes mast cell degranulation as an immunity response, and may cause release of transforming -growth- factor- β from ECM of fibroblasts, as activators for local stem cell lineage specification

8.1. Mast cells degranulation induced by acupuncture action

Mast cell densities were found to be higher around acupoints than nearby non-acupoints [127-131]. There is evidence that mast cells interact with connective tissue matrix components through integrins [132]. Interaction of nerve cells and mast cells [133-135] causes degranulation of the latter, releasing part (as a “piecemeal” release) or all of the large number of enclosed biochemical molecules which are involved in physiological and pathophysiological events [136]. These biomolecules include serotonin, proteases, heparin, granulocyte, macrophage, colony-stimulating factor (GM-CSF), leukotrienes, interleukins, transforming growth factors (TGF), tumor necrosis factor- α (TNF α), calcitonin, nerve growth factor (NGF), stem cell factor (SCF), substance P, histamine, prostaglandin, thromboxane, and other peptides like fibroblast growth factor (FGF) [45, 136, 137]. Such biomolecules, working separately or cooperatively, are involved in (1) allergy response, (2) acquired immunity, (3) innate immunity, (4) maintaining the life of sensory neurons, (5) inflammation, (6) supporting the growth of T cells and various tissues, (7) metabolic rate, (8) noxious stimuli response, (9) blood vessel tone regulation, (10) fibroblast growth, (11) wound healing, and (12) osteoblast formation.

There is direct evidence that acupuncture could cause degranulation of mast cells via mechanotransduction [130, 131]. Thus mast cells can be considered as mediators of the effector function of acupuncture. Note also that in classical physiology, mast cells are linked mainly to allergy response. However, as pointed out in [136], “why does an extraordinary cell type spend its life from the bone marrow through the circulation system, to the skin and lungs

aveola and just waiting for a few allergy antigens?" Also, according to more recent acupuncture research, why do more mast cells migrate along channels correlating with the TCM meridians? [39] Why are there so many "biomedical ingredients" or contents inside this cell type? [137] The interesting issue is: a mast cell degranulates under certain conditions (like increase of tension of collagen fibers attached to ECM of mast cell, via acupuncture) as a "grand sacrifice for life" and the empty shell seems to recycle after use and the story repeats [136]. I conjecture that, in the future, bridging acupuncture research and biomedical research of the functions of mast cell subset MC_{tc} (here the subscripts t, c stand for tryptase and chymase respectively), which resides in the connective tissue, would give clues to extensive answers of the above interesting questions, relating closely to immunity, wound healing and other physiological aspects.

8.2. Unfolding of the fibronectin (Fn) fiber fibrils associated with fibroblasts could activate and release transforming growth factors during wound healing, with stem cells specification playing a plausibly crucial role

Mesenchymal stem cells (MSC) are far away from their original source (bone marrow) and are "wandering" near various tissues/organs in the CTIF system for repair after injury, reinforcement of growth when necessary [138]. Upon activation, they differentiate into skeletal myocytes, smooth muscle cells, adipocytes, osteoblasts, chondrocytes and even neurons [139] and the substrate's mechanical property like elasticity is one key factor that decides which phenotype each stem cell is developed into [140]. It has been found also [141] that TGF- β can activate the differentiation of these adult stem cells. Since the transforming growth factor molecules are secreted (by various cells, including degranulation of mast cells) into the CTIF system in the dormant/latent state [142] in the midst of glycoproteins (fibronectin, laminin, ...), it is intriguing to ask under what conditions will the TGF- β molecules be activated. We have already discussed the evidence of cell-mediated unfolding of the Fn fibrils and the subsequent exposure of cryptic binding sites of blood vessel cells in Section 4 and [143, 144]. Now cellular Fn is synthesized by many cells, including fibroblasts, endothelial cells, chondrocytes, synovial cells and myocytes [145]. Some recent study [146] indicates that there are Fn cryptic binding sites which can be specifically bound by TGF- β . The above cited series of studies provide the possible/plausible linkage between mechanotransduction plausible arising from acupuncture, and the release of activated TGF- β in specification of stem cells for normal growth, repair/wound healing.

Along this line of logic, first note that latent transforming-growth-factor- β -binding protein (LTBPs) are ECM glycoproteins which play the major role of storing latent TGF- β in the ECM. In vitro study [147] indicated that Fn fibrils and their continuous presence were required for controlled storing of LTBP1 in the ECM of fibroblasts. Since TGF- β plays important roles in a wide range of cellular processes (including apoptosis and proliferation), it is important to find out whether Fn-activated TGF- β is controlled, at least in in vitro injury/healing model experiment. In fact, in a very recent study of liver injury, it has been shown that fibronectin protects excessive liver fibrosis by controlled local activation of TGF- β [148, 149]. The maneuvering process of acupuncture, either by piston-like action or rotation, auto-

matically extends the fibronectin network and tones the mechanical tension of other fibers, and that one consequence is the activation of TGF- β in the CTIF. The activated TGF- β molecules under the control of Fn fibrils may activate adult stem cells for proper healing [141]. Moreover, Fn matrices have been shown to act as storage of various growth factors which regulate cellular proliferation [72, 150]. Future research on whether acupuncture type of mechanotransduction would trigger release and/or activate some of these growth factors, for tissue/organ homeostasis, wound repair is an important project.

9. Discussions – Summary of the plausible consequences of acupuncture and some frontier issues in future acupuncture research

9.1. The emergence of the meridians as a natural system of communication of all multi-cell organisms

After a cell has completed its first mitosis into two and more cells, a system of communication must have existed to cater for the coordination of the function of a group of cells, and morphogenic development as a whole, before the nervous system is built up. Mechanical forces, including that derived from fluid flow in the embryo play crucial roles [151] in these functions. The mesoderm at a later stage develops mainly into the largest tissue of the body--the connective tissue system. Thus the CTIF system is a natural platform for communication, with the nervous system joining in at a later stage; those parts which can carry signals most efficiently, become the natural meridians channels. With the development of the blood and lymph circulations, nerve systems, immune system of various cells....., there are stations through which various types of signals can be sent/relayed with better efficiency to other parts of the body for survival of the organism. Acupoints have been hypothesized as such functional sites along the natural communication network [39]. Therefore, it is not too surprised to find from in vitro experiment that the CTIF network is mechanically connected once fibroblasts exert traction on the collagen fibers due to mechanotransduction, of which the action of acupuncture is one example. I would further note that the expression of the protein connexin 43 (Cx43) around acupoint ST36 and other acupoints along the stomach meridian was found to be higher than that of non-acupoints, suggesting that intercellular communication through the stomach meridian is more efficient than that through nonacupoint sites; see Section 2.3 & [152]. Moreover, two recent studies showed that manual acupuncture at ST36 influenced the pain thresholds of acid-induced and heat-induced pain in Cx43 heterozygous gene knockout mice [153, 154]. Having analyzed above, I would remark that some of the acupoints could also be morphological centers developed from embryo, as proposed in [155].

9.2. Summary of the plausible consequences of acupuncture action at a typical acupoint: acupuncture represents a micro-injury or micro-massage the consequences of which should be able to be explained by biomedical knowledge in principle

Referring to Fig.1, the number at each box indicates the section number for which the process is discussed. We start with the box 3.3, marked “acupuncture at acupoint”. This block joins via a solid arrow to the left box number 4.1, which represents fibroblasts cytoskeletal

remodeling. The solid arrow signifies that such a statement is suggested and supported by either in vitro or in vivo experiment. The dotted arrow towards another box is only speculative/hypothesized, without direct experimental support so far. An arrow together with a question mark from box 3.3 to box 9.5 indicates that, whether such a possible connection exists, is still an open question, awaiting further research to give clue to an answer. The dotted double-headed arrow joining boxes 7.3 and 9.5 represents a speculation that the two processes might be related. With some relevant basics introduced in Sections 2 & 3, following the discussions presented from Sections 4-8, the float chart is self-explanatory.

Acupuncture is a micro-injury with certain specific procedures. Manual acupuncture in action should in principle be able to be explained by evidence-based medical science. However, the effects of acupuncture as a whole are the complex physiological consequences of all types of mechanotransduction events (with different time scales) triggered by the needling action. Therefore, the acupuncture mechanism cannot be deduced by a simple sequence of experiments or a simple theory. Reviews bridging advancements in sections 1(B and C) might serve to help in probing the mystery existing over several thousand years. When we understand more about such “bridges” (of which this chapter is only one small example), we can attempt to explain step by step, but gaining progress continuously, more specifically the reasons behind the consequences of acupuncture.

9.3. The mystery of calcium concentration at acupoints

Employing the proton induced X-ray emission (PIXE) technique, the calcium content at various sites of the connective tissues of the lower limbs of 7 cadavers was measure [60]. The calcium concentration at a typical known acupoint was reported to be several tens higher than that of the non-acupoints which were along the same meridian, but was 100~200 times higher than that of non-acupoint, non-meridian sites. A needle shape electrode was built selectively catered for Ca^{2+} detection by another group [156]. The relative Ca^{2+} concentrations were found to be higher at five acupoints, as well as two mid-points between two known adjacent acupoints of the ST meridian as compared to sites of control (certain distances away). Correlation between relatively high calcium concentrations and various acupoints (as compared to non acupoints) have also been reported by other groups [157-160]. Above stated research results suggest that there are (extra-cellular, Section 2.4) calcium stores at, or near the acupoint sites. Note that a number of acupoints are found in the periosteum [58]. I hypothesize that there are also high concentrations of calcium stores at or near these acupoints because from basic physiology, we learn that there is continuous calcium transport from the bone back to the circulation system, while new stem cells, i.e. osteoblasts are migrating along the periosteum to build new bone in our daily life [38].

Incidentally, it is amazing to find experimentally that in the biological world the calcium waves can be frequency-modulated or amplitude-modulated to transmit information and be detected in cells [161-163], similar to radio signal communication. Whether different frequency of manual and electrical acupuncture stimulus could be “decoded” in a quantitative way by relevant cells will be an interesting topic. Thus there are intriguing aspects relating calcium activity and acupuncture

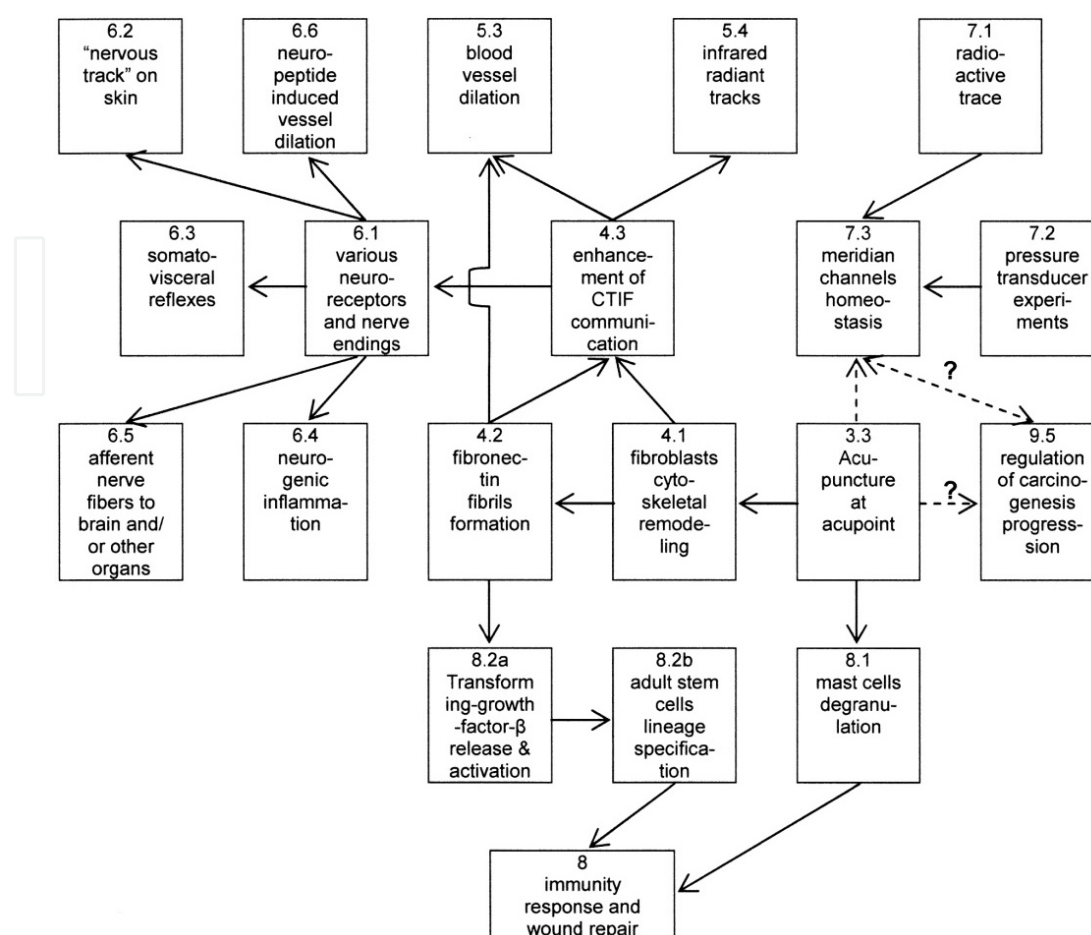


Figure 1. Some plausible biomedical consequences of acupuncture. Solid arrows represent deductions supported by either *in vivo* or *in vitro* experiments. Dotted arrows represent proposal/speculation, to be verified. Dotted arrow with question mark represents open questions. The dotted double-headed arrow joining boxes 7.3. and 9.5 represents a suggestion that two processes are related. The number in a box refers to the section number in the text where more details can be found. The basic of these processes are introduced in section 2 and 3.

9.4. The double-edge nature of nitric oxide and Hot/Cool concept in TCM in relation to the activation of different isoforms of the nitric oxide synthase

One very important function of Ca^{2+} is its combination with the calcium-calmodulin protein in the inner cell membrane to activate nitric oxide synthase (NOS) which catalyzes the substrate L-arginine to produce nitric oxide that can propagate through (lipid) cell membrane and through intra- and extra-cellular fluid. There are three isoforms of NOS, i.e., endothelial form eNOS, inducible form iNOS and neuronal form nNOS. The first one is active in endothelial and other cells to produce NO for (blood, lymph) vessel dilation and is continuously needed for living. The enzyme iNOS is activated when the immunity system is alarmed and produces a large amount of NO (with time scale around hours) that combines with superoxide in the mitochondria to form peroxynitrite (ONOO^-) which is supposed to be detrimental to membrane of alien cell. However, over expression of iNOS is very harmful to our body, playing important roles in various types of pathogenesis [164, 165]. Therefore iNOS inhibi-

tor can be used as drug to treat organ transplant rejection [166]. On the other hand, nNOS is expressed in neuronal cells and catalyzes (L-arginine) to produce NO also to act mainly as neural transmitter. Thus NO is a double-edge molecule: not enough NO from eNOS would cause hypertension; too much NO from iNOS would trigger a harmful auto-immune response (rejection of donor organ in case of transplantation); too much NO from nNOS would cause too much neural transmission, upsetting the nervous system. Too much iNOS activation may be associated to “Hot” type of disease in immune response; and too much nNOS activation may be related also to the “Hot” syndrome in nervous system; too much eNOS may be related to the “Cool” aspect of food causing hypotension, in the nomenclature of TCM. Whether a specific type of acupuncture modality would specifically enhance one specific isoform of the stated three enzymes is a challenging question which may or may not have a specific answer.

9.5. Can acupuncture modulate the routes of carcinogenesis?

Various cell types, including the epithelial cell, embryo fibroblasts, vascular smooth muscle cells, were cultured on Fn-coated hydrogel-based substrata which mimicked the physiological stiff CT. Starting from the basics of cell cycle analysis, it has been found that tension transmitted through integrin receptors to the cytoskeleton cooperates with the cyclins to control the G₁ phase of these cells types [167]. In another study, there is evidence that CT matrix stiffness controls the ability of ectopically expressed cyclin D₁ to go through the S phase entry during breast cancer progression [168]. Moreover, atomic force microscopy and fluorescence imaging in mice model indicates that comparable enhancement in arterial tissue stiffness occur at sites of cell proliferation in vivo [167].

On the other hand, TGF- β can regulate a diversity of cellular functions like proliferation, motility and differentiation with distinct specificity in different cell types. We have noted in section 8 that Mast cells liberate TGF- β during degranulation (e.g. via acupuncture) and unfolding of fibronectin could activate TGF- β which is often in its latent state. In most non-transformed epithelial cells, TGF- β induces apoptosis as a mean to avoid tumorigenesis. However, more rigid substrates in an in vitro study of epithelial cells treated with TGF- β induced epithelial-mesenchymal transition, leading to cell proliferation instead [169], showing double-edge nature. During the recent few years, a lot of works, with a few examples stated above, have been published on the profound influence of the mechanical properties of the ECM, together with the participation of proteins, cytokines on (organ/adherent) cell fate, carcinogenesis of tissues [170-173]. In all these studies, the stiffness of the ECM in the micro-environment is the major focus of attention. Note that in addition to TGF- β , other growth factors, like fibroblast growth factors (FGFs), the insulin-like growth factors (IGFs), and the epidermal growth factor (EGF) are also kept in the ECM of different cells; see discussion in [146]. Note that each organ is enclosed by a CT network and fibroblasts have abundant Fn in their ECM. That micro-mechanical stimulation or micro-massage from the action of acupuncture, on the ECM of the relevant cells might interfere the progress of carcinogenesis is not far-fetched. In fact, it has even been shown that the malignant phenotype of parenchymatous cells is reversible by correction of the altered cues of the ECM [174].

9.6. On “bio-thixotropy”, warm bianstone meridian-massage and moxibustion

For a homogenous substance, thixotropy may be considered as the ability of a semisolid gel to liquefy when stirred or shaken (or heated) and to return to its semi-hardened state after the external influence is taken away. Paint is one such homogeneous chemical example.

In the living world, and in the macroscopic scale, there was in vivo evidence of changes in short-range stiffness of muscles and tendons arising from compression force induced by a force transducer probe [175]. The result was interpreted as thixotropy property of muscles and CT building the tendons. There was also clear evidence that respiratory muscles of normal subjects also showed thixotropy property during respiration cycles [176]. Denaturation of type I collagen fibrils was reported to be a heat absorbing process accompanied by a change in heat capacity [177]. The fascia system was invoked also to possess thixotropy property to explain the beneficial effects of warm-up exercise and body massage [178]. I would remark that in view of the complicated structure of the CTIF stated above in this chapter, the CTIF is not a homogeneous system, and the “bio-thixotropy” property in the microscopic scale (< microns) has yet to be defined and to be analyzed with grater details in the future.

On the other hand, DNA, cell membrane, collagen fibers, even muscles are found to have properties of liquid crystals [179-181]; they can change phase subjected to change in temperature (plus other parameters). The “bio-thixotropy” property of living tissues could therefore occur upon change in phase of the living liquid crystals. In short, the collagen fibers in the CTIF would become more pliable, more liquid-like upon heating, for example, by warm bianstone massage along the meridians or moxibustion; note that bianstone acupressure was practiced even before needle acupuncture. Upon cooling, the collagen fibers would have been “toned”, as a speculation. It is also tempting to speculate that pretreatment with warm bianstone meridian-massage before needle acupuncture might provide a better platform of mechanotransduction along the meridian channels in the CTIF system.

9.7. Limitations of this chapter

(a) In this chapter, acupuncture, unless otherwise stated, refers to manual acupuncture only. Electro-acupuncture is outside the general scope of this chapter. The liquid crystal properties of living tissues certainly bring high complexity in electrical and optical communications within our bodies and deserve concerted effects of research in the future. (b) A number of plausible consequences are deduced based on in vitro experiments only here, with some verified by in vivo animal studies and / or clinical results. In order to expedite the accumulation of evidence-based knowledge, and progress of acupuncture research, this approach is inevitably workable according to the author, else we are leaving behind numerous results of in vitro experiments obtained in biomedical science research alone; these results are in fact related (though in many cases indirectly) to the consequences of acupuncture at this stage. (c) Other growth factors, in addition to TGFs are indeed kept in the CTIF system but have not been discussed here at this moment. Detailed consequences of mast cells degranulation, cells migration have not been analyzed here. Further review and research are necessary to bring in these aspects and might contribute further to understand better the consequences of acupuncture in action.

10. Conclusion – Acupuncture research is important for modern medicine, east and west

There are ex vivo and in vitro experiments to support the notion that piston-like or rotation type acupuncture at a typical acupoint activates the CTIF system which forms a mechanically well connected communication network. Recent in vitro experimental analysis indicates that mechanotransduction can be transmitted more efficiently along intra- and inter-muscular cleavage planes of the activated CTIF system, where most of the acupoints are found to be located, supporting the hypothesis [39] that the mechanical signals from acupuncture can be transmitted to the ECM of other residence cells (like mast cells) as well as ECM of organ/adherent cells (like cells of blood, lymph vessels, nerves). A large number of plausible biomedical events take place as explained in Sections 4-9; a schematic representation of some crucial events is presented in the flow chart of Section 9.2. Acupuncture is a micro-injury or micro-massage, the consequence of which should, in principle, be explained by evidence-based biomedical science. Therefore building bridges between results of the works stated in Section 1.2 (B and C) with reference to acupuncture research is important in modern medicine, east and west.

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