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REVIEW ARTICLE

Bonghan Circulatory System as an Extension of Acupuncture Meridians

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Abstract
The Bonghan system is a newly-discovered circulatory system, which corresponds to classical acupuncture meridians and was discovered in the early 1960s by Bonghan Kim. Despite its potential importance in biology and medicine, it has been ignored or forgotten for a long time. Only recently have most of its significant parts, such as the Bonghan system (BHS) inside blood or lymph vessels, on the surfaces of internal organs, and in brain ventricles, been confirmed. For this, novel methods using modern technology were necessary because Bonghan Kim did not describe his methods. For example, Among other methods, the discovery of a BHS-specific dye, trypan blue, was one of the most important original contributions that made BHS observation possible. With this technique, the BHS in adipose tissue became traceable, and the BHS was discovered on the fascia surrounding tumor tissues, a finding which may have great significance in relation to serious health problems in modern society, namely, obesity and cancer.

1. Introduction

The therapeutic effects of acupuncture are being increasingly accepted worldwide [1–3], and it is increasingly imperative to elucidate the mechanism of acupuncture's effects in terms of modern scientific concepts and terminologies. It must therefore be asked what is special about the acupuncture points (AP) and meridians (AM) and what distinguishes them from other neighboring areas of the skin. If there are special features, how does needling or other stimulation applied at APs function? To answer these questions, one must investigate the anatomical structure of APs and AMs.

Heine observed that at AP sites a composite of blood vessels and nerves existed within a sheet of loose connective mesenchyme perforating the superficial fascia that separates the subcutaneous from muscle tissue [4–6]. He demonstrated an AM-like structure for the fascia-myo-tendon chain of the lung AM [7,8], which were supported by other reports [9–12]. Structurally, APs are neurovascular bundles [13–16], neuromuscular attachments [17–20], and various types of sensory nerve endings [21–23]. Langevin observed that more than 80% of the APs and 50% of the meridian intersections of the arm appeared to coincide with inter- or intramuscular connective tissue planes [24–26]. Jones applied ultrasonic imaging to AP research [27], and Ifrim attempted to stain APs and AMs using Alcian blue [28]. A comprehensive review of the anatomic characterization of the acupuncture system may be found in the review by Van Wijk [29]. To the author's knowledge, no research has revealed any discrete

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anatomical structures corresponding to APs or AMs that are not known to Western biology or medicine. In this respect, Bonghan (BH) Kim's claim is unique in that it proposes the existence of a new circulatory system distributed throughout the body of not only humans, but of all vertebrae.

The National Acupuncture Meridians Research Institute, led by BH Kim, published a series of five reports on the anatomical structure and physiological study of APs and AMs [30–34] and one English review paper available in most university libraries [35]. A Bonghan corpuscle (BHC) and a Bonghan duct (BHD) correspond to an AP and an AM, respectively. BHDs are linked either to one end of a BHC or to both ends and collectively these structures form a novel circulatory system throughout an animal's body.

The Bonghan circulatory system is composed of several sub-networks located at various sites inside the body. These sub-networks can be categorized as: (1) a superficial BHC/D located in the skin, (2) an intravascular BHC/D that runs along the interior of the large veins, arteries, and lymphatic vessels and is afloat in the blood/lymph stream, not adhering to the vessel wall, (3) an extra-vascular BHC/D that runs along the exterior of large blood vessels, (4) an organ-surface BHC/D that spreads on various internal organ surfaces, (5) an intra-organ BHC/D located inside various internal organs, and (6) a neural BHC/D that exists inside the brain and spinal cord and runs along the exterior of peripheral nerves.

To examine the related physiological functions, first one needs to consider the liquid flowing in the BHD network. Analysis of BH liquid was performed by BH Kim [32], and important components include hyaluronic acid, neurotransmitter hormones such as adrenalin and noradrenalin, amino acids, and free nucleotides. The BH microcell or 'sanal' (formerly called granule) is a spherical or oval-shaped body with a diameter of 1–2 μm and containing one or two chromosomes enclosed by a thin membrane. BH Kim claimed that the 'sanal' played an important role in the regeneration of damaged tissues [33,34]. Another important function is the transmission of electrical signals through the BHD network, which can supply a structure-based mechanism for the well-known phenomenon of low electrical impedances at acupoints [36–38]. A hypothetical function of the BHD is light propagation, which may explain the almost instantaneous effects felt throughout the whole body with some needling at acupoints [39].

Until recently, BH Kim's claims could not be reproduced or confirmed mainly because the formula of the staining dye essential for tracing and identifying BHDs was undisclosed. Thus his work has been long neglected, and there has been no follow-up research except for the case of the Japanese anatomist Fujiwara [40] who was, in fact, able to partially reproduce BH Kim's results; however, his work also did not attract much attention.

To our knowledge, there has been only one serious histological investigation of APs that denied BH Kim's claims. Kellner thoroughly examined skin APs and concluded that no BHC-like structure existed [41]. However, one must be careful in drawing conclusions from a non-observation based on a histological method because a single histological method cannot fully reveal all structures in a tissue. In this case, it is essential to use a proper dye to visualize a novel structure like a BHC.

Since 2002, an intensive investigation of the BH system has been performed by the Biomedical Physics Laboratory, Seoul National University, and supported by the Korean Ministry of Science and Technology through the National Research Laboratory program. The first target for detection was the intravascular BHD in large blood vessels and lymphatic vessels of rabbits, rats, and mice. We then searched for the BHD on internal organ surfaces organs and inside brain ventricles and the central canal of the spinal cord. At present, a method to identify superficial BHDs and BHCs in the skin is still in development.

A series of investigations have been performed to establish the novelty of BHDs and BHCs and to elucidate their details. Besides conventional staining procedures and light microscopy, modern instruments and techniques unavailable in the time of BH Kim have been utilized. Confocal laser scanning microscopy [42], various types of electron microscopy, such as scanning electron microscopy (SEM), cryo-SEM, focused-ion-beam SEM, and high-voltage transmission electron microscopy (TEM) [43–45], X-ray microtomography [46], and atomic force microscopy [47] have been used to study ultrastructure. In addition, up-to-date technologies, such as fluorescent nanoparticles [48–50], immunohistochemistry [51,52], proteomic analysis [53], the ELISA technique for hormone analysis [54,55], and electrophysiological methods [56,57], have been employed.

2. Recent Studies on the Bonghan System

2.1. Intravascular BHD and BHC

BHDs inside the caudal vena cava of rabbits and rats were chosen as the first site for observing a transparent threadlike structure afloat in the blood stream. Intravenous injection of a 10% dextrose solution at the left femoral vein was the key technique developed by our team to replace blood with

a transparent liquid while retaining the BHD in the blood vessel for observation *in situ* with a stereomicroscope [58–60]. This technique had a very low success rate, even for a highly skilled microsurgeon, and was further confounded by a fibrin coagulation phenomenon, in which fibrin coagulation formed strings that could not be distinguished from a BHD by either a stereomicroscope or a phase-contrast microscope. Our original contribution here, which was not described in BH Kim's work, was that of finding a method to distinguish a BHD from the very similar fibrin strings. This technique used fluorescent staining with Acridine orange to reveal the rod-shaped nuclei that are hallmarks of the BHD, but are absent in fibrin [61,62]. Figure 1 shows a micrograph of the longest sample of an intravascular BHD from a rat artery ever obtained using a surgical method [63].

A surgical method involving the cutting of a piece of blood vessel and searching for a BHD in the specimen was not successful because the BHD apparently shrank and effectively disappeared. A liquid nitrogen quenching technique of a whole blood vessel was also not successful because it was not possible to identify a BHD in the cross section of a frozen blood vessel. In 2006, we devised a new method to reveal a BHD *in vivo* in the caudal vena cava of a mouse by injecting a staining dye, Alcian blue, into the femoral vein [64]. Another *in vivo* method developed to observe a BHD involved fluorescent microscopy and a fluorescent dye, Acridine orange, injected intravenously [65]. BHCs were also observed but required careful analysis for identification [66].

At present, these methods for observing intravascular BHDs are still not fully developed; skill and luck are needed to obtain the desired result. There is a need to develop a new variety of intravascular endoscope for observation of an intravascular BHD without staining.

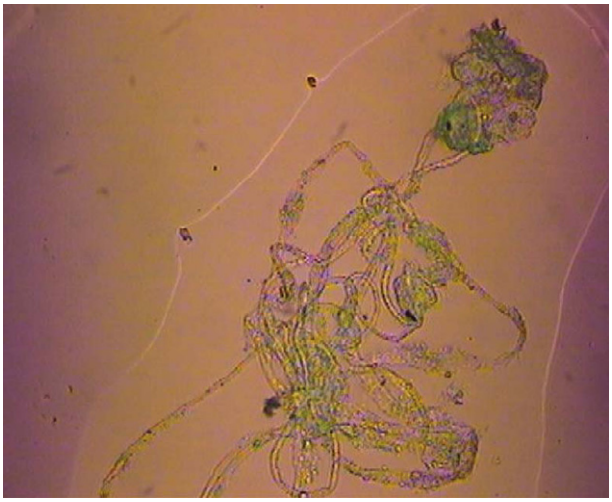
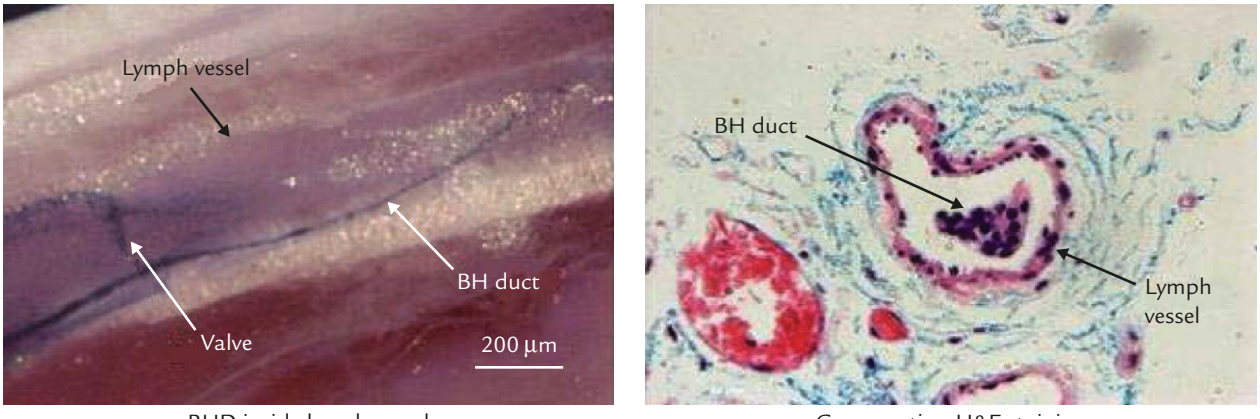


Figure 1 Phase-contrast microscopic image of a BHD from an artery of a rat. Total length ~4 cm [42].

2.2. Lymphatic BHD and BHC

As it is not possible to observe an intravascular BHD *in situ* in blood vessels because of the blood, it would be beneficial to examine transparent vessels, namely, lymphatic vessels. We attempted to detect BHDs in large lymph vessels using a stereomicroscope, but were only able to see lymphatic valves. We then injected several kinds of staining chemicals into a lymph vessel or node and were able to visualize BHDs *in situ* and *in vivo*. We found three different effective stains preferential for BHD over the surrounding lymph vessel: Janus Green B [67], fluorescent magnetic nanoparticles [48,49], and Alcian blue [46]. Figure 2 shows a lymphatic BHD, stained by Janus Green B, floating inside a rabbit lymphatic vessel.

The drawback to these three methods was the injection of chemical agents into the lymphatic vessels, potentially damaging the BHD or generating artifacts. A contrast-enhancing optical method was



BHD inside lymph vessel Cross-section H&E staining

Figure 2 BHD inside a rabbit lymphatic vessel stained with Janus Green B [43].

developed for *in vivo* observation of BHDs floating inside large-caliber lymph vessels [68] and we have successfully captured films showing the movement of a BHD as the animal respired.

2.3. BHD and BHC on internal-organ surfaces

A BHC/D network on the surfaces of various internal organs should be an easily confirmable structure, but several obstacles prevent easy observation. First, BHDs are thin and transparent and, thus hardly visible to the naked eye or under a low-magnification surgical microscope. Second, coagulated fibrin from blood present during surgery cannot be distinguished from BHDs. Third, similar-looking tissues from torn-off peritonea or capsules of internal organs are not easily discernable without histological examination. And lastly, there are difficulties in distinguishing BHDs from lymphatic vessels [42].

A distinguishing feature of BHDs is that they do not adhere to the surfaces or capsules of internal organs but move freely. In addition, a BHC may be doubly or multiply connected to BHDs. Important histological features include the presence of a bundle structure formed of several ductules and the distribution of rod-shaped nuclei aligned as broken lines. Extensive investigations on the morphological and functional nature of BHDs and BHCs on organ surfaces have been performed to elucidate their structural details and definitively establish their novelty [43–46].

2.4. BHD and BHC in the brain and spinal cord of rabbit

BHDs of 20–40 μm in diameter were observed floating in the cerebrospinal fluid of the brain ventricles and the central spinal canal of a rabbit. An effective *in situ* staining technique using hematoxylin was developed to visualize the BHD, and the presence of rod-shaped nuclei was confirmed by using various nucleus specific staining dyes [69]. Figure 3 shows the location of BHDs against the ependymal walls of a third brain ventricle and of the cerebral aqueduct in a rabbit.

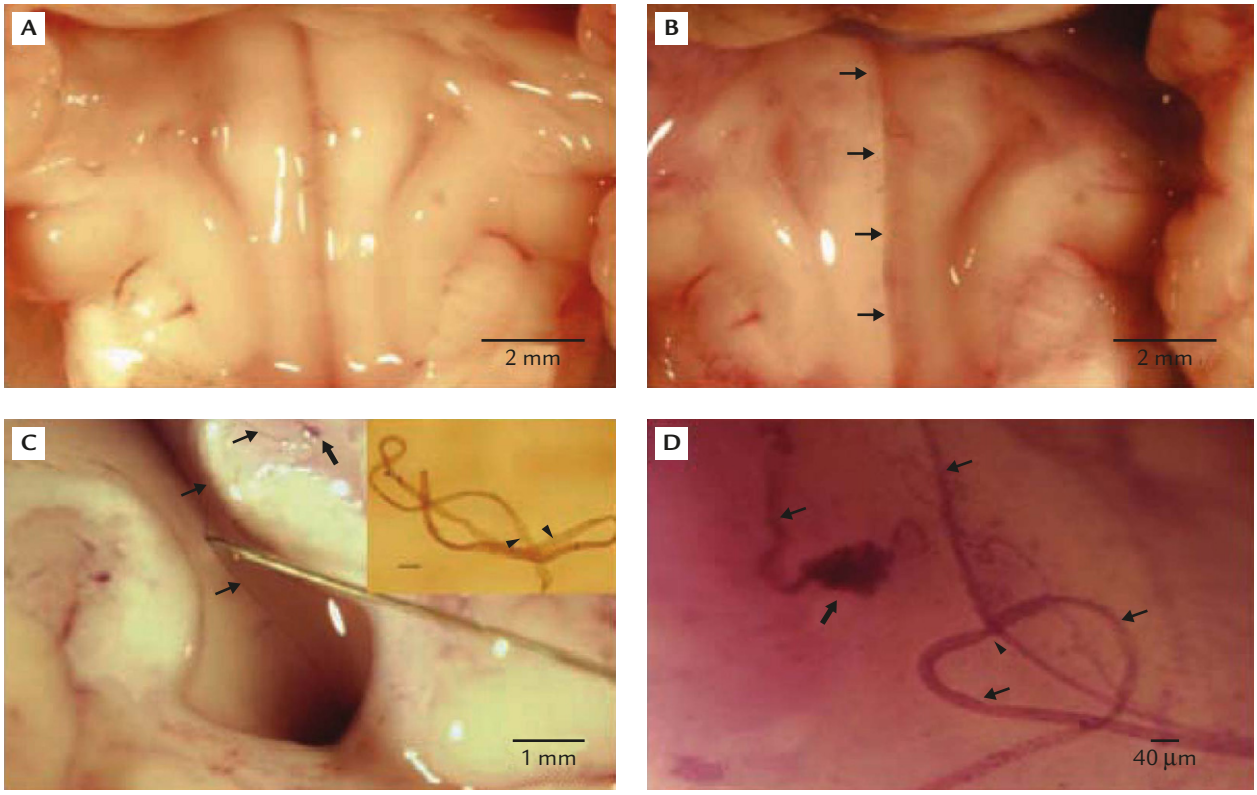


Figure 3 BHD in brain ventricles of rabbits. Stereomicroscopic images at bottom of the fourth ventricle beneath the cerebellum of same rabbit before, (A), and after, (B), hematoxylin application. No BHD visible in panel A but, after hematoxylin staining and washing, BHD (arrows) emerged near sulcus, panel B. (C) Stereomicroscopic image of BHD (arrow) in an aqueduct and third ventricle of rabbit brain after hematoxylin and washing, lifted using a needle to show it was a floating tissue in cerebrospinal fluid. Inset: wound state of threadlike structure specimen, showing its elastic nature; overlapped regions show its optical transparency; two nodes present (arrowheads); scale bar, 60 μm. (D) Stereomicroscopic image of BHD (arrow) with corpuscle (thick arrow) and node (arrowhead); one end of BHD cut at front part of third ventricle [50].

3. Trypan Blue as a BHD-specific Staining Dye

Finding the proper staining dye was the most critical factor in the rediscovery of the BH system. Without a proper dye, a target tissue will probably not be noticed, even under high magnification. The secret blue dye that BH Kim used when discovering the BHD network has not been identified [31,32]. We have found a variety of materials, such as methylene blue, methyl green, Janus green B, Alcian blue, hematoxylin, chrome hematoxylin, and fluorescent nanoparticles, to be only partially useful. Only very recently, we found an efficient staining dye, trypan blue, which preferentially stained BHDs rather than blood vessels, lymph vessels, nerves, muscles, or adipose tissues. Trypan blue is useful for vivistaining of vitreoretinal membranes in ophthalmic surgery [70] and is most commonly used to discriminate between live and dead cells. Strangely, it stained BHDs, but not other kinds of tissues, *in vivo* and *in situ*; thus it was extremely useful as a BHD-specific dye in detecting BHDs in various settings.

Using trypan blue, we were able to make significant new contributions, including finding for the first time weblike networks of BHDs on the omentum of a rat [71]. It is remarkable that the BHD-web overlaid the surface of the omentum and the peritoneum near the spleen (Figure 4).

As BHDs were often observed to enter adipose tissue around internal organs, we were not able to trace them into these tissues because the BHDs were not visible. BHDs and BHCs in adipose tissue can now be visualized with trypan blue [72], and the elucidation of their possible involvement with or relationship to obesity should be interesting. Most remarkably, we were able to detect BHDs on fascia surrounding tumor tissue using an *in situ* trypan blue-staining method. This will be discussed below.

4. Biological and Medical Significance

4.1. Circulatory function

The morphological requirement for the BH system to have circulatory function is the presence of channels in the BHD. This condition was confirmed using an H&E staining method [73] and various types of electron microscopy such as transmission electron microscopy (TEM), high-voltage TEM, Cryo-scanning electron microscopy (Cryo-SEM), and focused-ion-beam scanning electron microscopy (FIB-SEM) [43–45]. Cellular level evidence was the location of endothelial cells comprising the inner boundary of the ductules in the BHD, also confirmed by TEM [73], and an immunohistochemical study which supported the existence of endothelial cells [52].

A direct test to demonstrate liquid flow, performed by injecting fluorescent nanoparticles into an organ-surface BHC (Figure 5), revealed a one-way flow, as expected for a circulation system [74]. The average flow speed, recently measured by injecting Alcian blue into a BHC on the surface of a rabbit liver [44], was 0.3 ± 0.1 mm/s, in agreement with Bonghan Kim's data [32]. Liquid flow through a BHD from the skin toward the internal organs was observed by injecting chrome-hematoxylin and fluorescent nanoparticles in the skin near a rat testis.

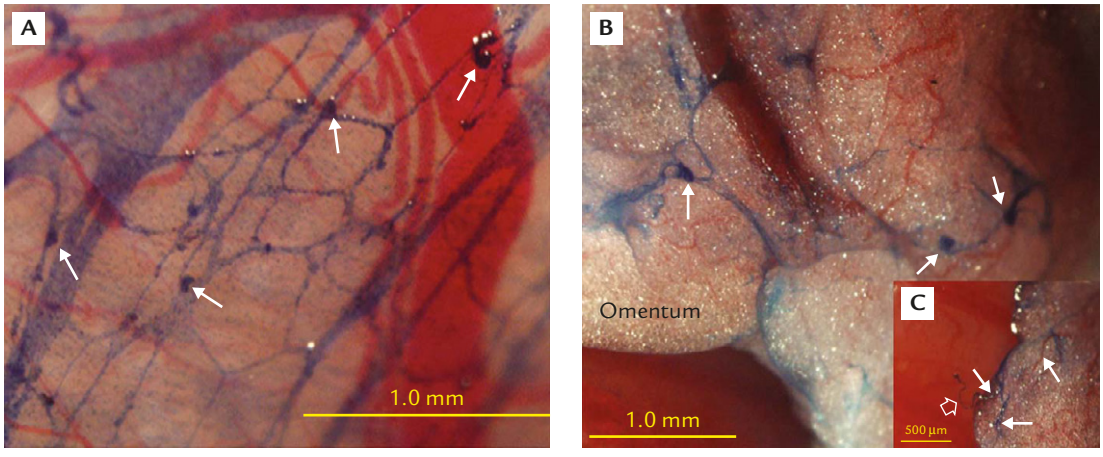


Figure 4 Weblike network of BHDs revealed by using trypan blue. (A) Web of BHDs on visceral peritoneum around stomach near rat spleen; several small BHCs at crossing points (arrows); blood capillaries not stained. (B) Network of BHDs on omentum below stomach and over small intestine; three small corpuscles at crossing points of BHDs (arrows). (C) Inset: another part of same omentum as (A); floating BHD (open arrow) connected to BHDs (arrows) in omentum, showing BHDs on omentum as part of larger network of freely movable BHDs on internal organ surfaces [43].

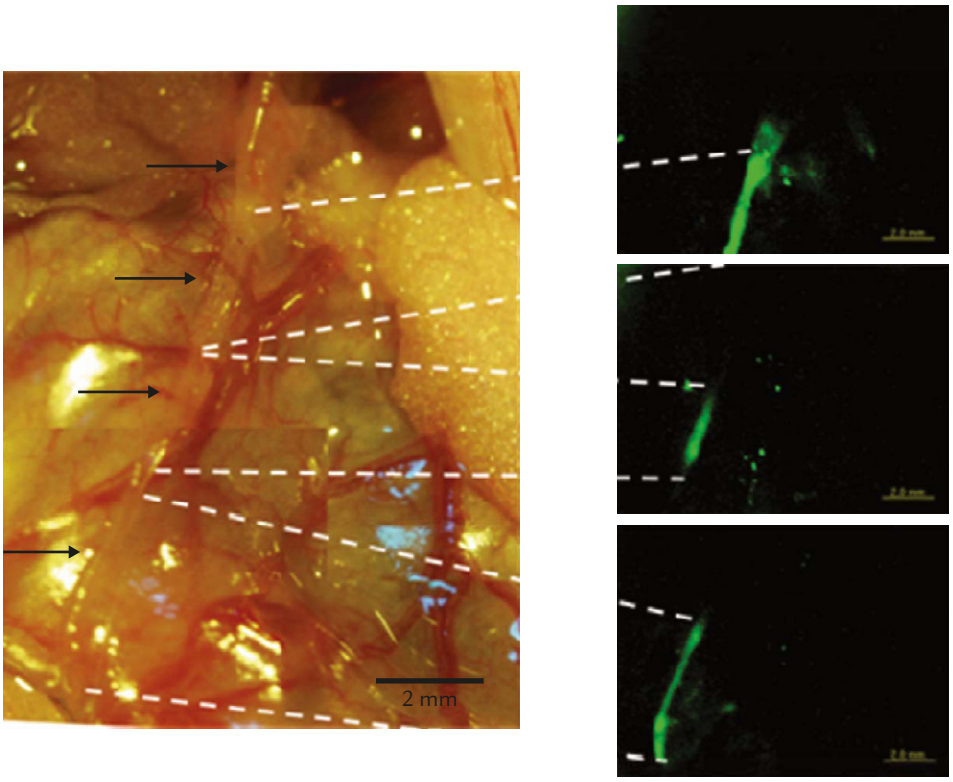


Figure 5 Left figure shows BHD (arrow) on rat small intestine. Right three panels show nanoparticle flow after injection at point indicated by top broken line. For first 4 minutes, nanoparticles in first region, then moved to middle after 12 minutes, and finally moved to third region in 18 minutes; speed 0.6 mm/min in only one direction [74]. Extremely slow speed due to conditions, such as temperature and humidity, and to time lapse from opening abdomen to injection of nanoparticles, about 60 minutes; peristaltic motion of BHD nearly stopped. With improved methods, including better viability conditions and shorter time lapse, we were better able to measure flow speed (0.3 mm/s) [44].

4.2. Electrophysiology: excitability

The circulatory function of the BH system also requires the existence of excitable cells, which can be tested using electrophysiological experiments. A BHC obtained from rat intestinal surfaces was placed in a Locke's solution bath and a microcapillary electrode inserted into the BHC cell membrane, which showed an abrupt electrical potential drop of 40 mV relative to the reference potential followed by irregular bursts of spontaneously evoked spikes in the resting potential at an average duration of 16 seconds (Figure 6). The resting potential and pattern of irregular bursts showed smooth muscle-like excitability of the cells in the BHC [56]. In addition, the irregular bursts showed some resemblance to certain firing patterns of neurons [75], which suggested a nerve-like electrical signal transmission in the BH system.

The nature of the excitable cells was determined by studying the effects of stimulation by acetylcholine and pilocarpine and the results showed hyperdepolarization, as has been observed in vascular smooth muscles. A critical factor at the molecular level of such a system is the Ca-ion channels that are necessary for cell movements in a contractible BHC. Testing with the Ca-ion-blocker nifedipine confirmed that the excitable cells in a BHC have Ca-ion channels [57].

In conclusion, electrophysiological results have supported the circulatory function of the BH system. The electrophysiology of the BH system could provide a scientific basis for widely used electroacupuncture therapy [76] and for the electrical properties of acupuncture points, which have been studied for many years worldwide [37,38].

4.3. Hormone path, immune function, and hematopoiesis

We measured noradrenalin (NA) and adrenalin (A) in the organ-surface BHC of a rabbit using the CatCombi ELISA kit and identified chromaffin cells that secrete NA and A [54,77]. The presence of chromaffin cells at the acupoint CV12 was also explored using a immunohistochemical technique,

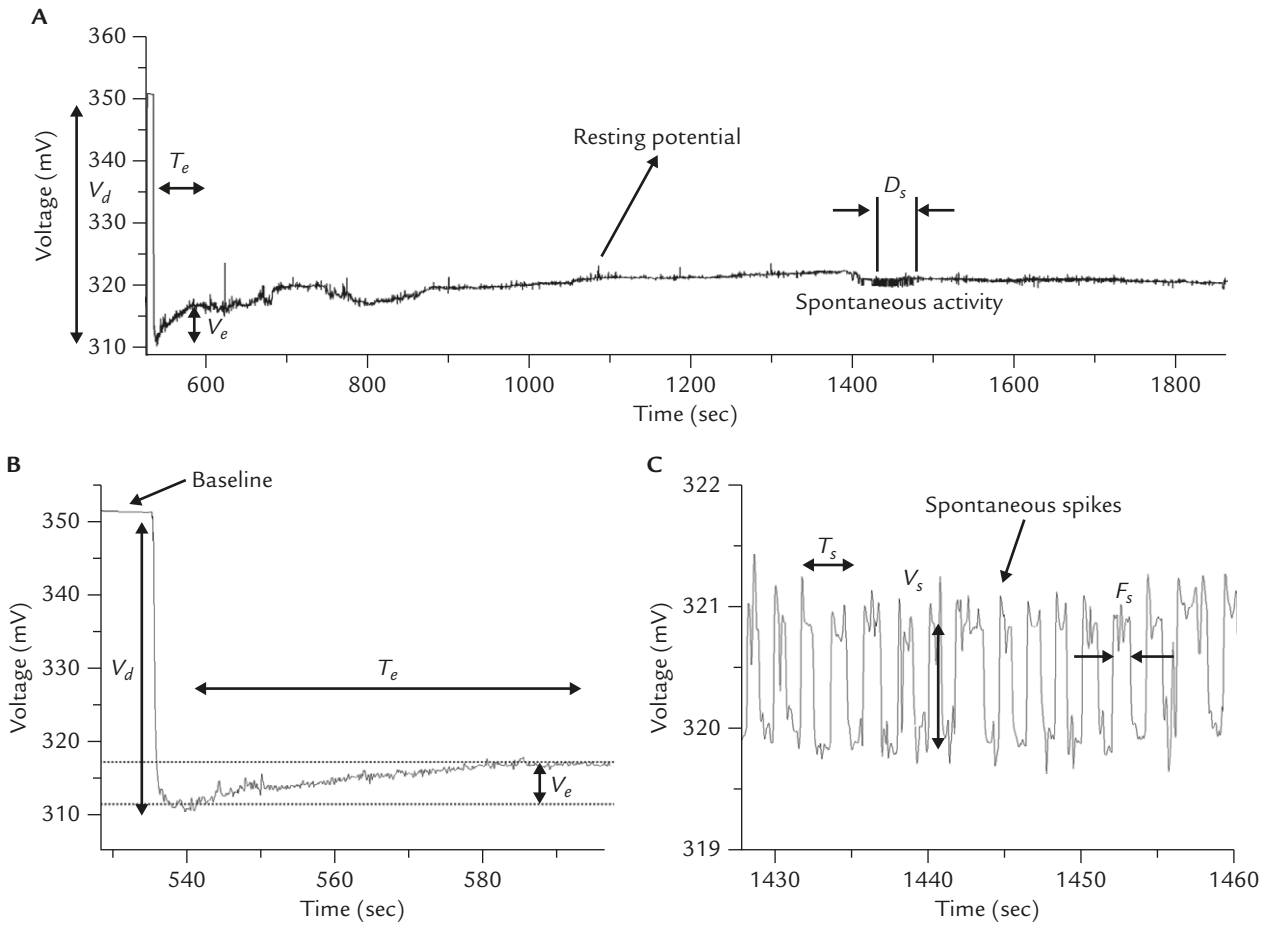


Figure 6 (A) Resting potential and spontaneous electrical activity of a cell in a BHC. (B) At the moment of microcapillary insertion into cell membrane of BHC, potential decreased abruptly by about 38±15.5 mV (n=11) from reference potential of bath; V_d is potential drop; potential increased slowly to resting potential of 10.5±8.4 mV (n=11); V_e is small increase (dotted line); and T_e is time of increase (18.1±14.0 sec (n=11)). Resting potential remained stable with fine background fluctuations; irregular activity of spontaneous spikes in resting potential arose for durations (D_s) of about 16.6±14.9 sec (n=11). (C) Spontaneous activity recorded in a period expanded in time and in voltage; average amplitude (V_s) was 1.2±0.6 mV (n=11) and average period (T_s) 0.8±0.6 sec (n=11); spikes had an average half-width (F_s) of 0.27±0.19 sec (n=11) [57].

yielding results consistent with BH Kim's claim [32,55]. These results provided a new view of acupoints as an endocrine catecholamine organ besides the currently-known adrenal medulla, post ganglionic fibers, and Merkel cells [78].

Improved immune function and beneficial effects on inflammation are often described after acupuncture treatment [2] and an abundance of mast cells is reported at acupuncture points [79]. We observed that the organ-surface BHC and BHD contained a significant number of monocytes, eosinophils, mast cells, and macrophages [43,45,73]. The abundance of such immune cells in the BHD supported evidence for the related therapeutic effects of acupuncture treatment and for BH Kim's claim that the organ-surface BHD is an extension of the classical acupuncture meridian system.

Blood cells are known to be generated in the bone marrow but BH Kim claimed that the intravascular BHD is another hematopoietic organ [34]. Indeed, we observed here that the BHD became thicker and thus easier to detect when anemia was induced by the injection of phenylhydrazine. Many red blood cells in early stages of maturation were observed in organ-surface BHCs when anemia was induced.

4.4. Regeneration and sanal (microcell)

Regeneration of damaged liver cells was reported in BH Kim's fourth article [33]. Considering this claim, we hypothesized that there might be adult stem cells in BHCs and to verify this hypothesis, we stained sliced BHCs and BHDs with stem cell marker antibodies. We observed that mesenchymal

stem cell (MSC) markers were strongly expressed in a manner similar to bone marrow. Extracellular matrices were also consistent with stem cell expression [52].

Proteomic analyses of the tissues and liquid from the BHD on rabbit intestinal surfaces indicated the existence of proteins related to the recruitment of MSCs [80], the cell processes in MSCs (ezrin, actinin, and myosin) [81], and the differentiation of MSC/myofibroblasts (alpha-smooth muscle actin and CD147) [82]. These protein profiles suggested that BHDs located on organ surfaces have roles as temporary depots and points of differentiation of stem cells for tissue regeneration.

Damaged liver tissues are regenerated by the gathering of sanals that had migrated through the BHDs [33]. This process has not been specifically investigated here, but some basic studies have been performed on BH microcells, revealing that their motion appeared to be Brownian, but that they also showed some peculiar light interactions. Their average speed was not affected by visible light, but was significantly increased by UV-A (360 nm) [83,84]. The presence of DNA inside a sanal was identified using various types of DNA-specific staining, such as Feulgen reaction [42], DAPI, and PI, and the state of the DNA shown to be fragmented by a TUNEL assay [85].

The detailed surface morphology of sanals was studied by topographic imaging and error-signal imaging from atomic force microscopy, their mechanical properties investigated by force modulation microscopy, and their electrical characterization determined by electrostatic force microscopy [47]. Further investigations of the morphology of the budding sanals have been performed by SEM and atomic force microscopy [86]. Measurement of the membrane's elastic Young's modulus indicates that sanals have much harder membranes than similar-sized apoptotic bodies [87].

4.5. Obesity and cancer

Obesity is one of the major health problems in modern societies. Incidentally, BHDs and BHCs are well developed in adipose tissues and have been visualized using trypan blue, (Figure 7) [72]. Apparently, stored fats and the BH system have various relationships which require more detailed future investigation.

Buikis et al observed that microcells in certain tumor tissues grew rapidly and transformed into young undifferentiated cells and called this cytological mechanism for the immortality of tumor cell populations "sporosis" [88]. We conjecture that their microcell-sporosis is actually nothing less than one of the 'sanal' proliferation processes that normally occur in the BH system [33,89]. Such conjecture was strengthened by proteomics data [53], showing an abundance of carbohydrate-based processes associated with stem cells [90,91], cancer cells [92], and differentiated myeloid cells [93].

A direct relation of the BH system to tumor tissues has indeed been found. Cancer cells were subcutaneously injected into a nude mouse and BHDs and BHCs observed in the fascia wrapping tumor tissue that grew in the skin [94], visualized by using the trypan blue method (Figure 8). We hypothesize that, besides the well-known blood and lymphatic routes, BHD connected to cancer tissue is a novel metastatic route. In such case, the BH system may potentially play a double role: a path of metastasis

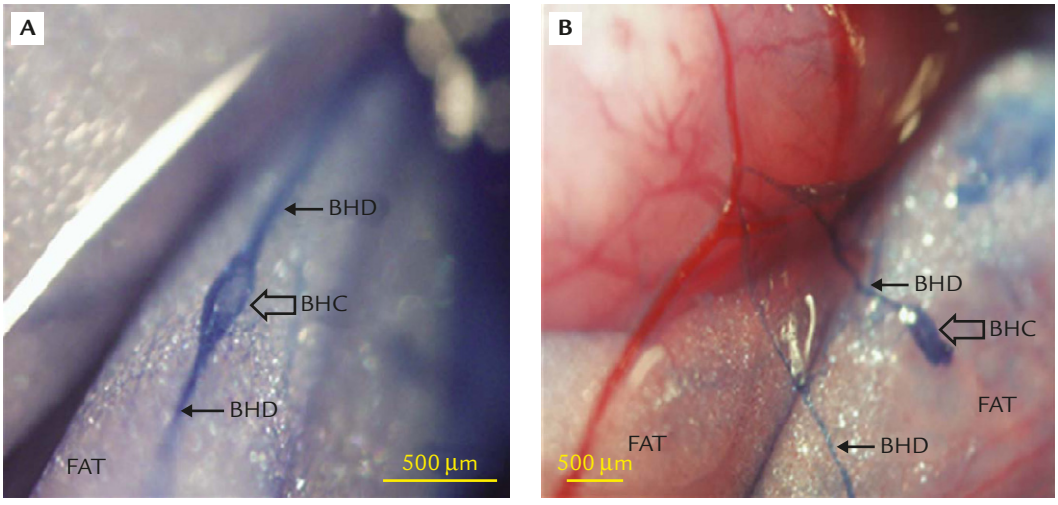


Figure 7 Trypan blue staining of BHD and BHC inside adipose tissues. (A) BHC and connected BHD inside adipose tissue around rat small intestine. (B) BHC and two BHDs near same rat small intestine; blood vessels and adipose tissues not stained [71].

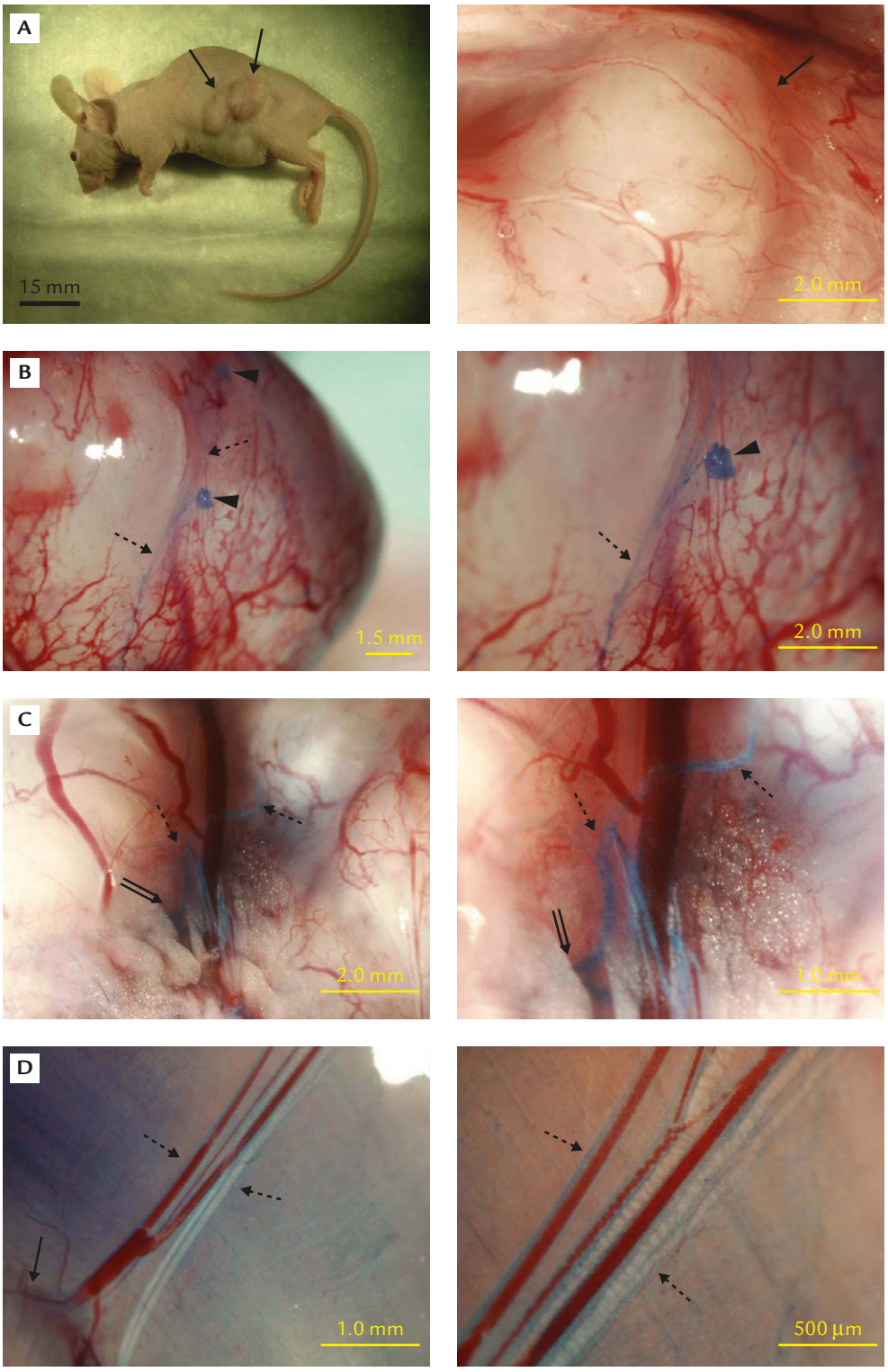


Figure 8 Visualization of a Bonghan system on fascia surrounding tumor tissue in mouse skin. (A) Images of tumor tissue (arrows); left, image of mouse with two tumor tissues grown for 2 weeks after subcutaneous inoculation with human lung cancer cells; right, part of tumor tissue surface after skin resection; presence of Bonghan system hardly noticeable. (B) *In-situ* trypan blue staining revealed Bonghan ducts (dotted arrows) and corpuscles (arrow heads) on tumor tissue fascia; right panel, magnified view of left panel; trypan blue did not stain blood vessels. (C) Sample showing multiple Bonghan ducts (dotted arrows) on tumor tissue fascia surface; right panel, magnified view of left panel, showing branching of Bonghan duct which notably, enters nearby fat layer (double arrow). (D) Trypan blue technique revealed Bonghan ducts (dotted arrows) along bundle of blood vessels and nerves; right panel, magnified view clearly showing duct along blood vessel; bundle of blood vessels and nerves connect tumor tissue (arrow) at lower left corner to outside skin. Samples A, B, C, and D from different mice [94].

as well as a means to control tumor tissue by acupuncture treatment, because the BH system is an extension of the acupuncture meridian system.

5. Discussion

Some frequently raised questions are discussed here with brief answers. Any further questions are welcome. The email address is kssoh1@gmail.com. The homepage is http://kmc.snu.ac.kr.

5.1. Intravascular Bonghan duct

(1) Why was the intravascular BHD (IBHD) not observed or noticed by surgeons or by researchers of blood or blood vessels?

As a matter of fact, threadlike structures are commonly observed in blood-vessel-opening events, but may have been mistaken for fibrin strings. In a surgical situation with open blood vessels, fibrin strings form with coagulated blood and look similar to and are hard to discern from an IBHD, even with ordinary or phase-contrast microscopy. To make matters worse, fibrin has a strong affinity for IBHD and enshrouds it upon severe blood vessel damage. Our group's original contribution here was the development of Acridine orange fluorescence imaging to reveal the nuclei distribution and clearly distinguish the IBHD from fibrin strings (Figure 9).

(2) Why is the IBHD difficult to observe *in situ* and *in vivo*?

The IBHD is hard to observe because it is a thin (~20 μm diameter), transparent, threadlike structure floating inside an opaque blood stream, thus requiring a staining technique for visualization. Here, we devised an *in vivo, in situ* observation method employing Alcian blue injection [64], but the success rate was quite low and depended upon experimenter skills.

(3) How did BH Kim discover the IBHD?

According to his report, he injected a certain blue dye at an acupoint and later observed the IBHD *in vivo* and *in situ* in large vessels, suggesting that he not only found the IBHD but also established the circulatory function of the acupuncture meridians and the IBHD. However, he did not present other salient details on the method or materials and, since then, no one has reproduced his method. Here, our team has only been able to stain IBHD by injecting Alcian blue into a blood vessel, not the acupoints.

(4) What are the functions of the IBHD?

According to BH Kim's claim, the principal role of IBHDs is hematopoiesis, but there may be other circulatory functions for important biochemicals such as hormones and tissue-regeneration materials. Kim also claimed that, in the early stage of chicken egg development, the IBHD was formed first; later, blood vessels formed around the IBHD.

(5) In which blood vessels are IBHDs observed?

IBHDs are observed in large arteries and veins, inside the heart, and in large lymph vessels. In this laboratory, IBHDs were detected in an abdominal artery and vein, femoral veins, hepatic veins, veins to kidneys, and large lymph vessels near the caudal

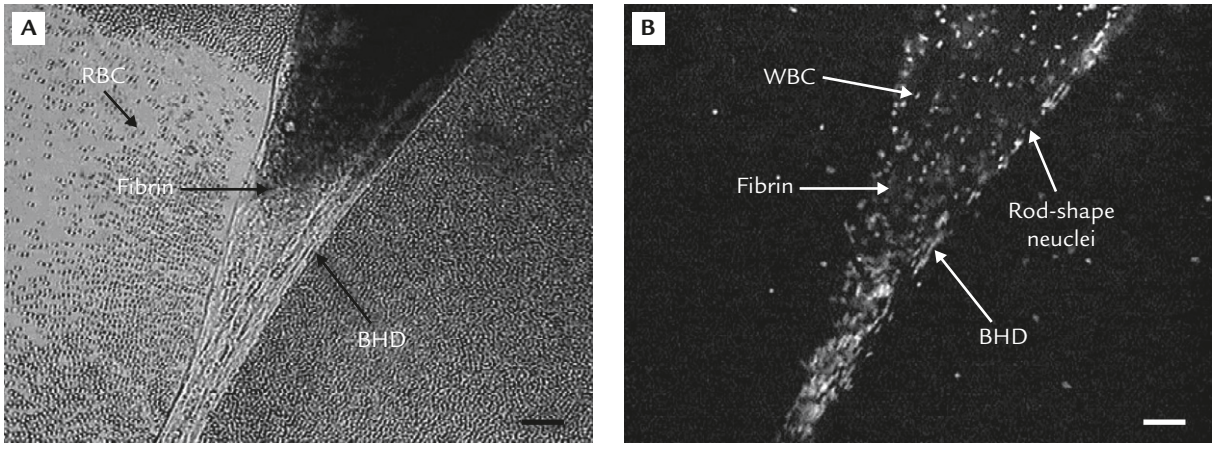


Figure 9 (A) Threadlike structure with enshrouding fibrin observed on a slide by differential interference contrast microscopy. The fibrin and the BHD were hardly distinguishable and red blood cells scattered around; scale bar, 50 μm. (B) Threadlike structure with enshrouding fibrin observed using Acridine orange fluorescence method; scattered dotted points, white blood cells; long rod-shaped nuclei from threadlike structure; clearly distinguishes fibrin from BHD; scale bar, 50 μm [65].

vena cava. IBHDs are supposed to be connected to other extra-vascular BH ducts and to go out from the vessels through vessel walls, a rare observation [68]. IBHDs did not appear to exist in capillaries or small lymph vessels. The subject animals were rabbits, rats, and mice.

(6) What would happen to the IBHD in surgery?

The IBHD is an elastic tissue and, once broken, it can coil and shrink. During any surgery, the IBHD would perforce be broken and could grow rapidly to restore the network. If the IBHD is not restored for some reason, the recovery from surgery may be hampered or some side effects might persist. Neither BH Kim nor anyone else, however, addressed the recovery process.

5.2. Acupuncture and the superficial Bonghan system

(1) Why is it difficult to observe BHDs corresponding to acupuncture meridians?

The size of a BHD is not very small (~30 μm diameter), but staining is required for detection in either *in situ* or a histological specimen. For example, in skin a host of lymph capillaries exists, yet it was not possible to visualize these until relatively recently. Without visualizing agents, histological examinations show no lymph capillaries in the skin. Likewise, without a visualizing agent, BHDs are hard to detect and, until now, a staining dye specific for the BHD was not known.

(2) What about BH corpuscles? Since the diameter of a BH corpuscle is ~1.0 mm, would it not be missed if a suitable microscope were used?

We examined a skin specimen including an acupuncture area and a histological study using the usual 10 μm thickness H&E (hematoxylin and eosin) staining did not reveal any noticeable structure. This technique required skill and an enormous amount of time and labor because a 1 mm thick specimen yields 100 sections to examine. At least 5–10 mm thick (width) skin would be required not to miss the acupoints. Furthermore, artifacts were easily introduced such that only trained observers with knowledge of the sought-after structure were able to discern the desired structure from the artifacts or other known structures. Therefore, we could not pursue this semi-thin section technique and, without semi-thin light-microscopic images, it was virtually impossible to apply ultra-thin (10 nm thick) TEM. Instead, we needed to develop a new method using thick sections (100–150 μm) with immunohistochemistry and multi-photon confocal laser scanning microscopy (MPCLSM). Using this method, we could visualize a plexus of blood vessels and nerve endings at the acupuncture points, but BHDs were not visualized because antibodies for BHDs are not yet known or available.

(3) What is the critical feature in identifying the BHCs?

One critical and characteristic feature is the presence of chromaffin cells near the center of the BHC. These cells secrete adrenalin (A) and noradrenalin (NA), whose principal endocrine tissues are the adrenal medulla and the postganglia of a nerve. The presence of chromaffin cells was reported by BH Kim and we were able to confirm the presence of chromaffin cells in a BHC on the surface of an internal organ and in the acupoint CV (conceptual vessel) 12 of rabbits [55].

Another feature of BHCs is a surrounding smooth muscle outer layer. We are currently performing experiments to visualize this layer, which would define the BHC as a separate skin structure.

A third feature is a bundle of a BHD and blood vessels attached at the bottom of a BHC and connected to neighboring BHCs. Detailed identification of such BHDs requires TEM imaging, which, in turn, will be realizable with accumulation of sufficient experience with BHCs.

5.3. What is a simple criterion to distinguish a BHD from other similar looking transparent threadlike structures?

A simple, but effective, criterion is to observe the distribution of nuclei in threadlike structures. The nuclei were rod-shaped and distributed in aligned broken-lines (Figure 10).

5.4. What are the histological features that distinguish a BHD from a lymph or blood vessel or a nerve?

The histological characteristics and ultrastructural features are compared in detail elsewhere [73]. In short, a BHD is a bundle of many tubules with interspersed fibrin fibers while a blood or lymph vessel is a single tube whether it is large or small in size.

5.5. What is an easy technique to detect a BHD *in situ* on fascia surrounding various tissues such as liver, stomach, intestine, heart, brain, or tumor tissues?

An easy technique for detecting a BHD is to spread and wash trypan blue on the surface of the fascia, allowing BHDs to emerge as blue-colored threads [71].

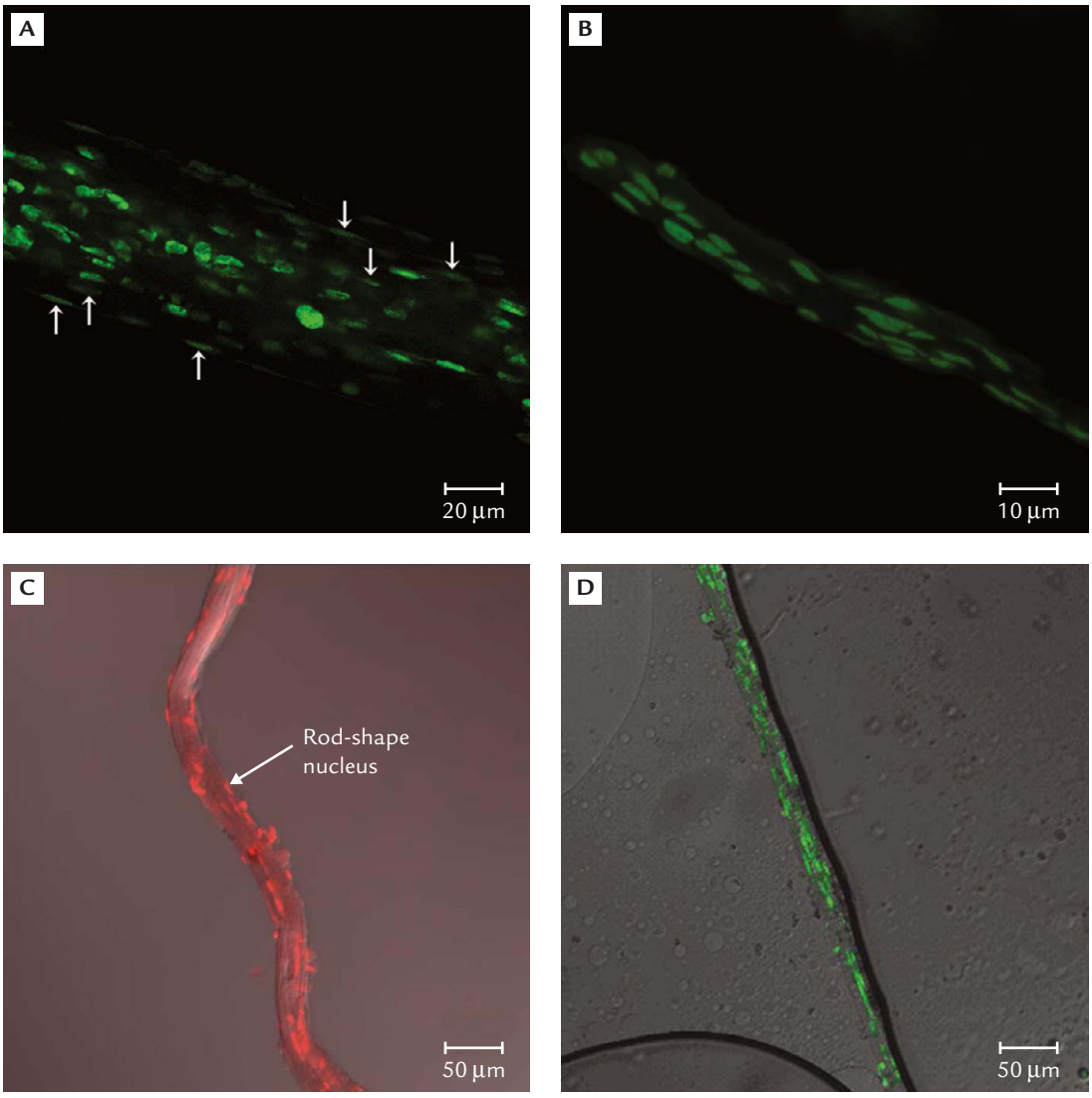


Figure 10 Confocal laser scanning microscopic images of BHDs showing rod-shaped nuclei (arrows) distributed in broken-lined striped fashion. (A) BHD stained by YoYo-1, a DNA-specific dye, after removal from Alcian-blue-injected rabbit lymphatic vessel [46]. (B) BHD stained by Acridine orange, a DNA-specific dye, after removal from a Janus-Green-B-injected rabbit lymphatic vessel [67]. (C) BHD stained using a Feulgen reaction, a DNA-specific dye, after removal from the rabbit organ surfaces [42]. (D) BHD was stained by Acridine orange after removal from rabbit caudal vena cava [61]. Shapes, lengths, and distributions of rod-shaped nuclei similar to each other in all four cases, suggesting that BHDs in lymph vessels, in blood vessels, and on organ surfaces belong to same system.

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