



RESEARCH ARTICLE

Comparison of the Characteristic Features of Bonghan Ducts, Blood and Lymphatic Capillaries

Vyacheslav Ogay¹, Kyung Hee Bae¹, Ki Woo Kim², Kwang-Sup Soh^{1*}

¹Biomedical Physics Laboratory, Frontier Physics Research Division and Center for Theoretical Physics, Seoul National University, Seoul, Korea

²National Instrumentation Center for Environmental Management, College of Agriculture and Life Sciences, Seoul National University, Seoul, Korea

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Abstract

Objective: To show that the characteristic morphological and ultrastructural features of a Bonghan corpuscle and duct presented here are consistent with the description given in the early reports of Bonghan Kim.

Materials and Methods: We compared the morphological aspects of Bonghan ducts with those of blood and lymphatic capillaries on the ultrastructural level to display the manifestly distinctive nature of the Bonghan system.

Results: The walls of the ductules were observed to be composed of a single layer of endothelial cells with characteristic rod-shaped nuclei and were not surrounded by a basal lamina or by accessory cells, such as pericytes or smooth muscle cells. The abluminal cell membranes of Bonghan ductules were not attached by anchoring filaments to the fibers of extracellular matrices as observed in lymphatic capillaries. The cytoplasmic processes of ductule endothelial cells appear to form overlapping and interdigitated interconnections which completely lack junctional elements. Although the cytoplasm of ductule endothelial cells contained a well-developed rough endoplasmic reticulum and many free ribosomes and polysomes, there was a relatively small number of pinocytotic vesicles and lacks specific organelles, such as Weibel-Palade bodies.

Conclusions: The Bonghan corpuscles are specialized structures consisting of different types of immune cells randomly scattered as single cells in the matrix or clustered in follicle-like formations. Moreover, the Bonghan ductules in the corpuscle contain flowing immune cells and occasionally basophilic bodies.

1. Introduction

A new circulatory system consisting of Bonghan ducts (BHDs) and Bonghan corpuscles (BHCs) was first found in 1963 in rabbits and other animals by the

North Korean scientist, Bonghan Kim [1]. According to his description, BHDs and BHCs are divided into two main types, depending on their location in the body: superficial (in the skin) and profound (deep in the body). Superficial BHCs are at acupuncture

*Corresponding author. Biomedical Physics Laboratory, Frontier Physics Research Division, Seoul National University, Seoul 151-747, Korea.
E-mail: kssoh1@gmail.com

points, as complex tissue formations with a bulbous shape surrounded by a dense net of blood capillaries, and interconnected with BHDs, specialized tubular structures comprised of a bundle of several small tubes or the ductules. Based on histological observations, Kim concluded that ductules were formed by a thin layer of endothelial cells with characteristic rod-shaped nuclei.

A similar histological structure was also observed in profound BHDs, which were found in peritonea, on the surfaces of various internal organs, in the brain, and inside blood and lymphatic vessels. They were semitransparent or milky-colored threadlike structures that were clearly different from peripheral nerves and blood and lymphatic vessels. Profound BHDs were linked with profound BHCs, which present as tissue formations of an oval- or cucumber-like shape and contain chromaffin cells and many immune cells. Bonghan Kim claimed that superficial and profound BHCs and BHDs formed a complex circulatory system responsible for the therapeutic effects of acupuncture. Based on histological and physiological results, he suggested that the BH system was the anatomical basis for acupuncture points and meridians in both humans and other mammals [1,2]. His report drew much attention from both Western and Eastern countries, but no one could fully reproduce his results [3–7]. For this reason, his studies were met with skepticism and have been long neglected.

Recently, interest in this new circulatory system, the so-called Bonghan (BH) system, has been increasing worldwide, including in China [8] and the United States [9]. Independent research teams in Korea, in addition to the group at the Seoul National University, are rapidly growing in number [10]. From the early stages of Bonghan theory in the 1960s, the most frequent question or criticism was that Kim may have mistaken lymphatic vessels for BH ducts [4,5]. This question has been addressed by the demonstration of anatomical differences between these structures [11] and by the observation of the BH system inside large-caliber lymph vessels [12–15]. Nevertheless, the structures of BH corpuscles and ducts still need further characterization to distinguish them from blood or lymph vessels.

The current work is a descriptive article to provide illustrations of the morphological characteristics of BH corpuscles and ducts, with particular emphasis on their differences from blood or lymphatic vessels. In 2002, an intensive reinvestigation of the BH system was launched by the Seoul National University group led by Soh with funding provided by the National Research Laboratory program of the Korean Ministry of Science and Technology. As a result of more than 6 years of intense work, many aspects of the BH system have been confirmed by

employing modern techniques and introducing new methods. As Kim's articles did not describe his methods, developing original methods and equipment was essential in this research and some results were derived using novel methods.

Intravascular BHDs inside blood vessels were the first target for confirmation [16,17], and we developed a novel method to distinguish BHDs from similar-looking fibrin strings [18,19]. The ability to distinguish between a BHD and a fibrin string was an essential technique, comprising an original contribution, and explaining why other researchers and experts in surgery have not noticed the presence of intravascular BHDs. Recognition of intravascular BHCs required further careful processes [20]. BHDs and BHCs inside large-caliber lymphatic vessels were visualized using three different staining agents [12–15] and were observed without staining by devising novel, contrast-enhancing, optical devices [21]. BHDs were also confirmed to exist in brain ventricles and in the central canals in rabbit spinal cords [22].

BHDs and BHCs on the surfaces of internal organs were extensively investigated with instruments and techniques not available to Bonghan Kim. Confocal laser scanning microscopy [11], high voltage transmission electron microscopy (EM), field ion beam scanning EM, cryo-scanning EM [23,24], X-ray microtomography [15], fluorescent nanoparticle techniques [13,14], immunohistochemical techniques [25], and proteomic analysis [26] were utilized for these investigations. Measurement of the liquid flow speed in BHDs was performed by injecting Alcian blue; the speed of travel was rather slow, at 0.3 ± 0.1 mm/sec [24]. The electrophysiological properties of BHCs showed a smooth muscle-like excitability that supported a putative circulatory function for the BH system [27]. Researchers have also confirmed that BHCs had contained chromaffin cells that produced and stored catecholamine, suggesting the medical significance of the BHDs as a hormonal pathway [28].

DNA-containing bodies flowing in BHDs have been collected and examined by electron [29] and atomic force microscopy [30]. These bodies (formerly called granules or sanals) displayed active chaotic motion whose speed was affected by 360 nm UV light [31]. The viscosity of the flowing liquid in BHDs has been measured [32].

In spite of significant progress in elucidating the properties of the BH system, the degree and details of the differences between the BH and the lymphatic systems still remains a controversy to many not familiar with the details of BH theory. The purposes of this instructive article are to demonstratively display the histological and ultrastructural BHD and BHC features which were found in agreement

with Bonghan Kim's original description [1], and to clearly show the ways in which BHDs differ from lymphatic or blood vessels.

2. Materials and Methods

2.1. Animals

Female New Zealand white rabbits (aged 8–10 weeks) were obtained from the Hanlym Laboratory Animal Company (Seoul, Korea) and housed in a temperature-controlled environment (23°C) with 60% relative humidity. All animals were exposed to a 12 hour light-dark cycle and provided food and water *ad libitum*. Procedures involving animals and their care conformed to institutional guidelines and in full compliance with current laws and policies (*Guide for the Care and Use of Laboratory Animals*, National Academy Press, 1996).

2.2. Anatomical observation

Rabbits were deeply anesthetized with urethane (1.5g/kg) and the abdominal wall opened. The Bonghan ducts and corpuscles on the surfaces of internal organs were visualized by staining with 1% methylene blue dissolved in physiological saline. The Bonghan ducts and corpuscles were then rinsed several times in saline and observed under a stereomicroscope (Olympus SZX12, Japan). The images were captured with a CCD camera (Olympus DP 70, Japan).

2.3. Light microscopy

Bonghan corpuscles and ducts were fixed in 10% NBF (Neutral Buffered Formalin) for 12 hours at 4°C, rinsed in PBS (phosphate buffered saline), dehydrated in a graded ethanol series, clarified in xylene, and embedded in Paraplast (Sigma, USA). Paraplast sections, 8 µm in thickness, were next stained with hematoxylin-eosin (H&E) for general morphological observation, mounted with Neomount, and examined with a light microscope (BX51, Olympus, Japan). Images were acquired with Image Pro Plus Software.

2.4. Transmission electron microscopy

For TEM examination, tissues were fixed with 2.5% glutaraldehyde in a 0.1 M sodium-cacodylate buffer at 4°C for 4 hours, postfixed in 1% OsO₄ in a 0.2 M sodium-cacodylate buffer each for 1 hour, dehydrated with ethanol and propylene oxide, and embedded in epoxy resin (Epon 812). Ultrathin sections were collected on large scale copper grids, contrasted using 2% uranyl acetate and Reynolds' lead

citrate, and examined in a transmission electron microscope (JEM 1010, JEOL, Japan) at an accelerating voltage of 80kV, with images obtained using a digital camera (ES1000W, Gatan, USA) and software processing (Gatan, USA).

3. Results

3.1. Anatomical and histological observation of Bonghan corpuscles and ducts on internal organ surfaces

Images of the anatomical locations of a BHC and BHDs in a rabbit body revealed that BHC was located in the peritoneum of internal organ surfaces, such as the large and small intestines (Figure 1C). A BHC was a small elongated tissue of milky color, measuring a few millimeters in length, and linked to thin semitransparent BHDs, themselves difficult to discern with the naked eye or without staining with methylene blue (Figures 1A, 1B). In addition, BHCs and BHDs were found located in the peritoneum, but not attached to the internal organ surfaces.

The histological structures of BHCs and BHDs were examined by stained thin cross-sections with H&E and light microscopic observation (Figures 2, 3). Histologically, a BHC tissue contained different cell types, such as monocytes, granulocytes, and small and large lymphocytes [23,24], scattered randomly in the matrix as single cells or gathered in a follicle-like formation (Figure 2A). The BHC surface was not surrounded by a distinct connective tissue membrane or capsule (Figure 2F) and, inside or near follicle-like formations, there were several small channels or ductules. The ductule walls were formed by a thin single layer of endothelial cells and ductule diameters ranged from 7–15 µm, large enough to transport liquid and/or accommodate a single immune cell (Figures 2B, 2C). In addition, H&E staining revealed that some ductules contained small basophilic bodies (~1 µm, Figures 2D, 2E) apparently composed of significant amounts of basophilic structures containing nucleic structures, such as chromatin [11,29], which were strongly stained by hematoxylin [30–32]. It should be also noted that, in the histological sections, no erythrocytes were observed in the ductules.

In comparison to BHCs, the histological structure of BHDs appeared simpler, with a BHD formed from a bundle of several ductules exhibiting characteristic rod-shaped nuclei (10–20 µm in length) that were clearly visible by phase-contrast microscopy (Figure 3A). In cross-section, the BHD presented as a small tissue formation containing several small lumens, 6–10 µm in diameter (Figure 3B). The lumen of the

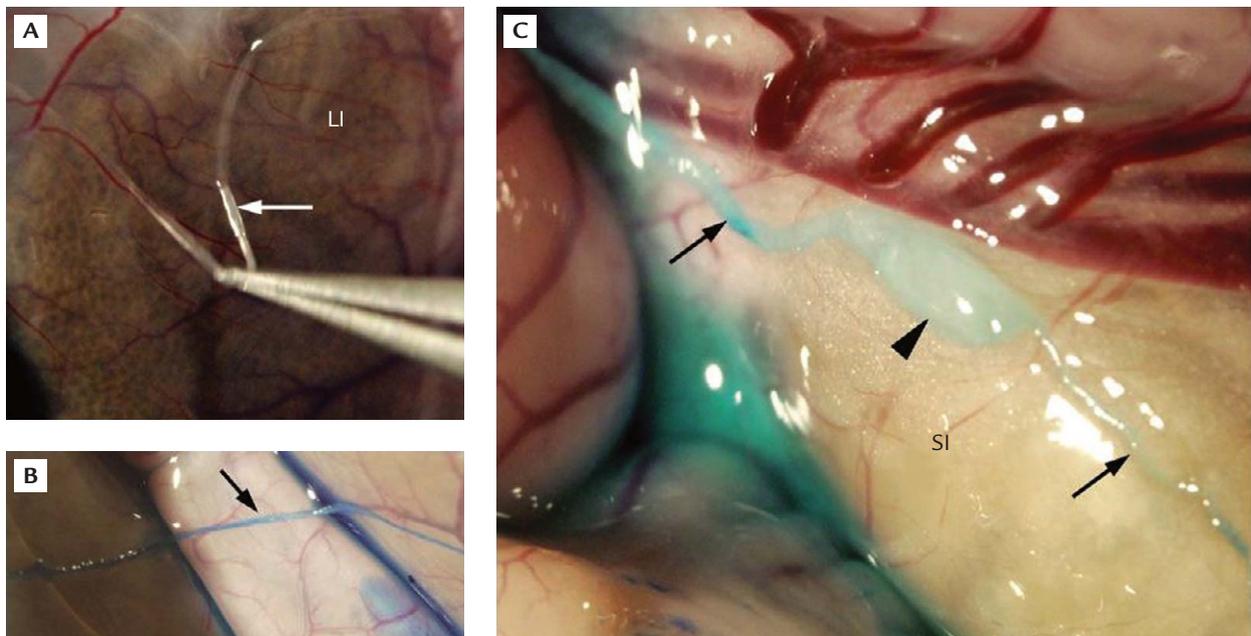


Figure 1 Stereomicroscopic images of Bonghan ducts and corpuscle on the surface of rabbit internal organs. (A) Bonghan duct (arrow) on large intestine surface (LI); intact duct, a semitransparent, freely movable tissue structure. (B) Bonghan duct (arrow) after methylene blue staining. (C) Bonghan corpuscle (arrowhead) on small intestine (SI) linked with Bonghan ducts (arrows); corpuscle and ducts contrasted using methylene blue.

ductule comprised a single layer of endothelial cells surrounded by an extracellular matrix.

3.2. Ultrastructural morphology of the Bonghan ductules, blood and lymphatic capillaries in rabbit

A comparative morphological study at the ultrastructural level was performed to characterize ductules in detail and to differentiate them from blood and lymphatic capillaries. Electron microscopy revealed that the ductules in a BHD shared some common properties with blood and lymphatic capillaries, but possessed their own distinct structural features (Figures 4–6). Similar to lymphatic and most blood capillaries, a ductule was made up of a single, non-fenestrated endothelial cell layer (Figure 4) and had a relatively small, regular lumen, $\sim 6\mu\text{m}$ in diameter, similar to a blood capillary. However, a lymphatic capillary possessed a more irregular and wider lumen than a ductule or a blood capillary and its endothelium was extremely attenuated (Figure 6A). In comparison with blood and lymphatic capillaries, which were surrounded by continuous and discontinuous basement membrane, respectively (Figures 6C, 6E), the abluminal wall of the ductule was not lined by an enveloping membrane (Figures 4C, 4D). Another specific morphological feature which differentiated ductules from lymphatic capillaries was that the ductule's abluminal wall was not connected

to interstitial collagen fibers by anchoring filaments composed of elastic fibers (Figures 4, 6). Instead, the ductule was surrounded by fibrin-like fibers, which were randomly distributed in the stroma of the BHD (Figures 4, 5). In the cytoplasm of ductule endothelial cells (DECs), a well-developed rough endoplasmic reticulum (RER) and relatively large vacuoles were distinctly visible (Figures 4C, 5C). Besides the numerous ribosomes that were distributed as beads on the membranes of the RER, many were free ribosomes and polysomes. However, the amount of pinocytotic vesicles or caveolae in DECs was small in comparison with blood endothelial cells (BECs) and lymphatic endothelial cells (LECs) (Figure 4D). Like LECs, the cytoplasm of DECs also lacked cell-specific organelles, such as Weibel-Palade bodies, usually present in the endothelium of blood vessels and capillaries [33]. The continuity of the endothelial lining was provided by interdigitated and overlapping DEC cytoplasmic processes (Figures 4B, 5B) that did not form characteristic intercellular adherens junctions, such as desmosome-like structures and tight junctions which usually were found in BECs and LECs (Figures 6B, 6E).

4. Discussion

In this paper, we provide images that clearly demonstrate the description of BHDs and BHCs on the

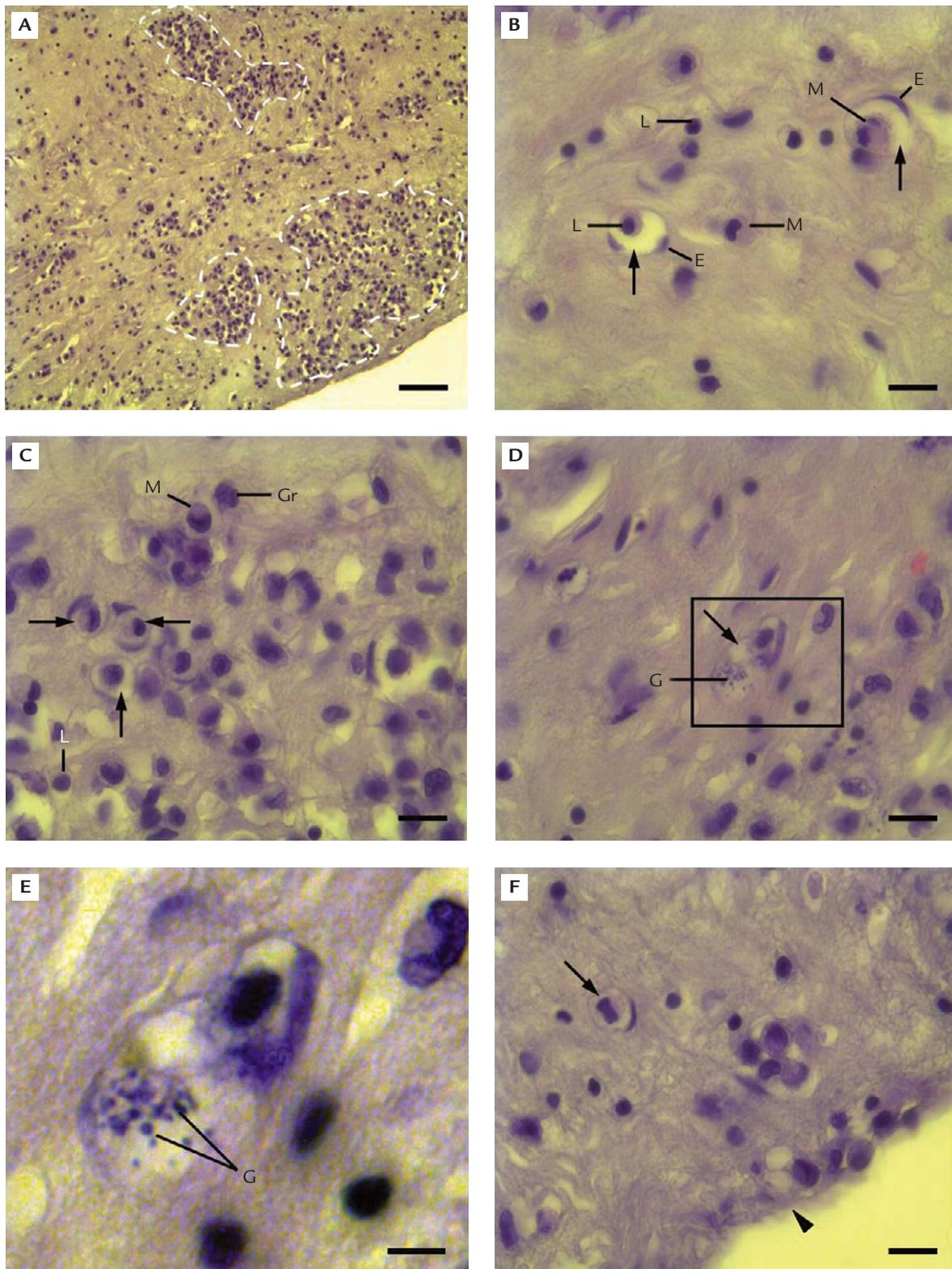


Figure 2 H&E stained cross-sections of a profound Bonghan corpuscle. (A) Light microscopic image of histological structure of corpuscle; low magnification; dashed line areas indicate gathering of different cell types, similar to follicular formation; scale bar, 50 μ m. (B) Details of ductules (arrows) in corpuscle; scale bar, 10 μ m. (C) Ductules (arrows) between follicle-like clusters of immune cells; scale bar, 10 μ m. (D) Small basophilic bodies (G) in the ductule (arrow); scale bar, 5 μ m. (E) Enlargement of area in rectangle of Figure 2D; scale bar, 5 μ m. (F) Photomicrograph of boundaries (arrowhead) of corpuscle not surrounded by external connective tissue membrane; "E" = endothelial cell; M = monocyte/macrophage; Gr = granulocyte; L = lymphocyte; scale bar, 10 μ m.

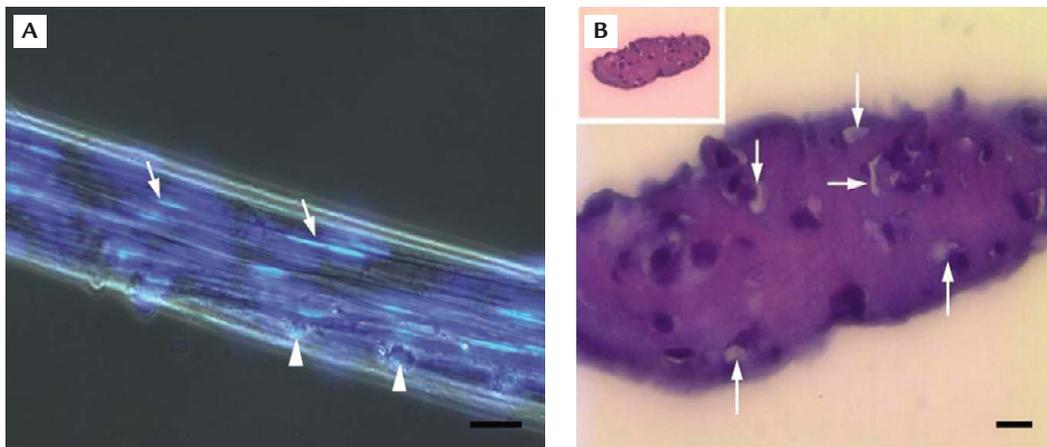


Figure 3 Histological structure of the Bonghan duct. (A) Photomicrograph of merged phase-contrast and fluorescent images of a duct; duct formed by bundle of several ductules (arrows) with characteristic rod-shaped nuclei stained with DAPI (blue); immune cells (arrowheads) on duct surface; scale bar, 50 μm . (B) Photomicrograph of several ductules in cross section through duct; small insert, general histological view of duct; scale bar, 10 μm .

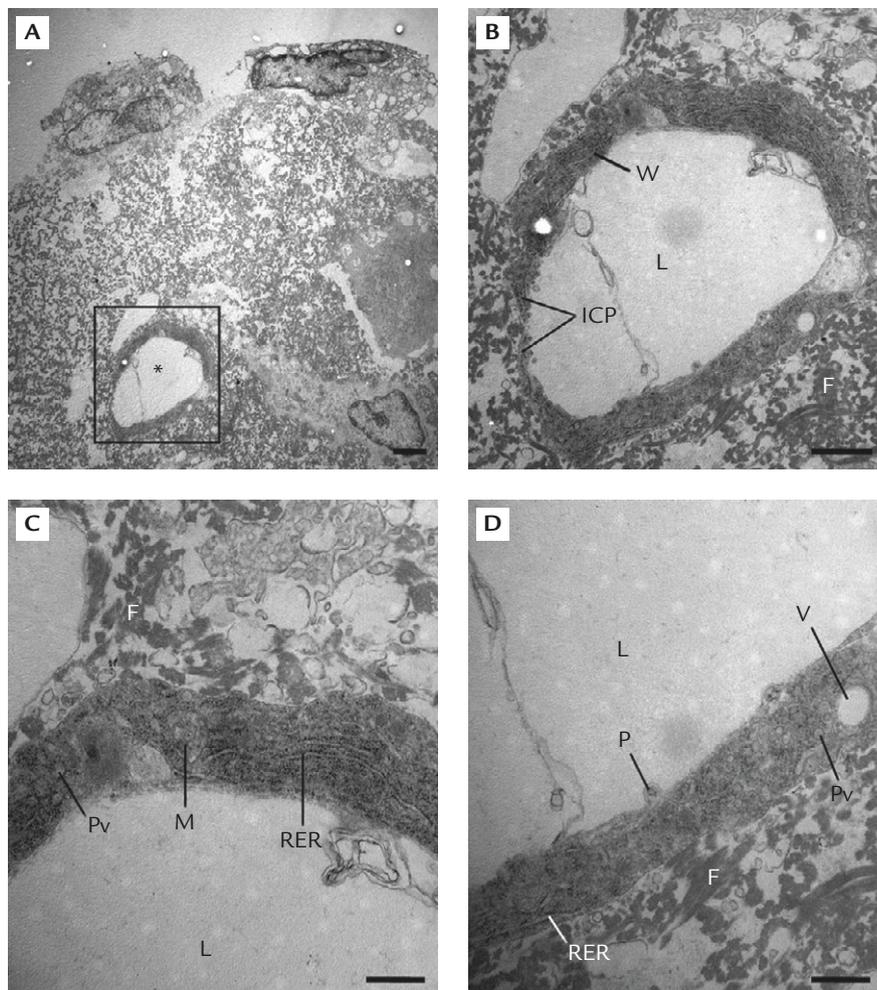


Figure 4 Ultrastructural organization of the ductule. (A) Electron micrograph of ductule (asterisk) cross-section; scale bar, 2 μm . (B) Magnified image of rectangular area in Figure 4A showing wall (W) of ductule as single layer of endothelial cells surrounded by fibrin-like fibers; scale bar, 1 μm . (C, D) High magnification EM showing ultrastructural characteristics of ductule endothelial cells; L=lumen; M=mitochondria; RER=rough endoplasmic reticulum; P=cytoplasmic protrusion; ICP=interdigitated cytoplasmic processes; Pv=pinocytotic vesicles; V=vacuole; and F=fibrin-like fibers; scale bar, 0.5 μm .

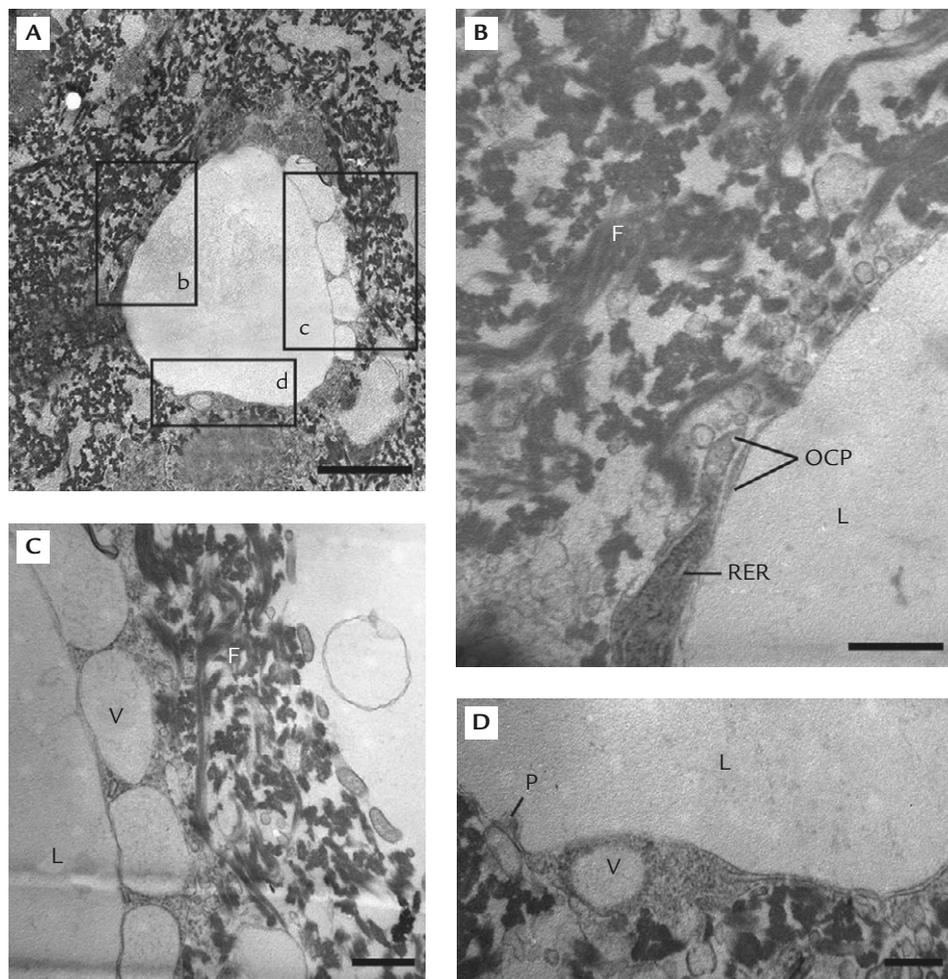


Figure 5 Microscopic view of the ductule. (A) Low magnification EM of duct cross section; scale bar, 2 μm . (B–D) Magnified images of rectangular areas in Figure 5A showing ultrastructural features of ductule endothelial cell; L=lumen; RER=rough endoplasmic reticulum; OCP=overlapping cytoplasmic processes; P=cytoplasmic protrusion; V=vacuole; F=fibrin-like fibers; scale bar, 0.5 μm .

surfaces of rabbit internal organs, as described previously by Bonghan Kim [1]. A detailed microscopic analysis with light and electron microscopy was performed to characterize specific anatomical, histological, and ultrastructural features of BHCs and BHDs. A BHD consisted of a bundle of several small tubular structures or Bonghan ductules (Figure 3), whose walls were formed by a thin single layer of endothelial cells possessing characteristic rod-shaped nuclei. Moreover, the same kind of Bonghan ductules were also found in the BHC, a type of expanded structure located irregularly along the Bonghan duct.

Our histological observations revealed that BHCs contained randomly scattered single cells in the matrix or gathered in follicle-like formations, suggesting that BHCs somewhat resemble a lymphoid organ within which immune cells reside and develop. It is plausible that macrophages, granulocytes, and lymphocytes in the follicle-like clusters could contact

each other and present antigens, thereby stimulating an immune response. Immune cells and occasionally basophilic bodies were visible in the lumina of Bonghan ductules.

Although Bonghan ductules share some properties with the endothelium of blood and lymphatic capillaries, they have distinct structural characteristics at the ultrastructural level (Table, Figure 7). First, in comparison with the morphology of a lymphatic capillary, Bonghan ductules observed here had a relatively small and regular lumen formed by a single layer of endothelial cells. Second, in contrast to blood and lymphatic capillaries, Bonghan ductules were not surrounded by a basal lamina or by accessory cells, and the abluminal cell membrane of a ductule was not attached to extracellular matrix (ECM) fibers as in lymphatic capillaries (Figure 7) [34,35], where they preserve the functionality of the lymphatic vessels and capillaries when interstitial pressure rises by preventing vessel

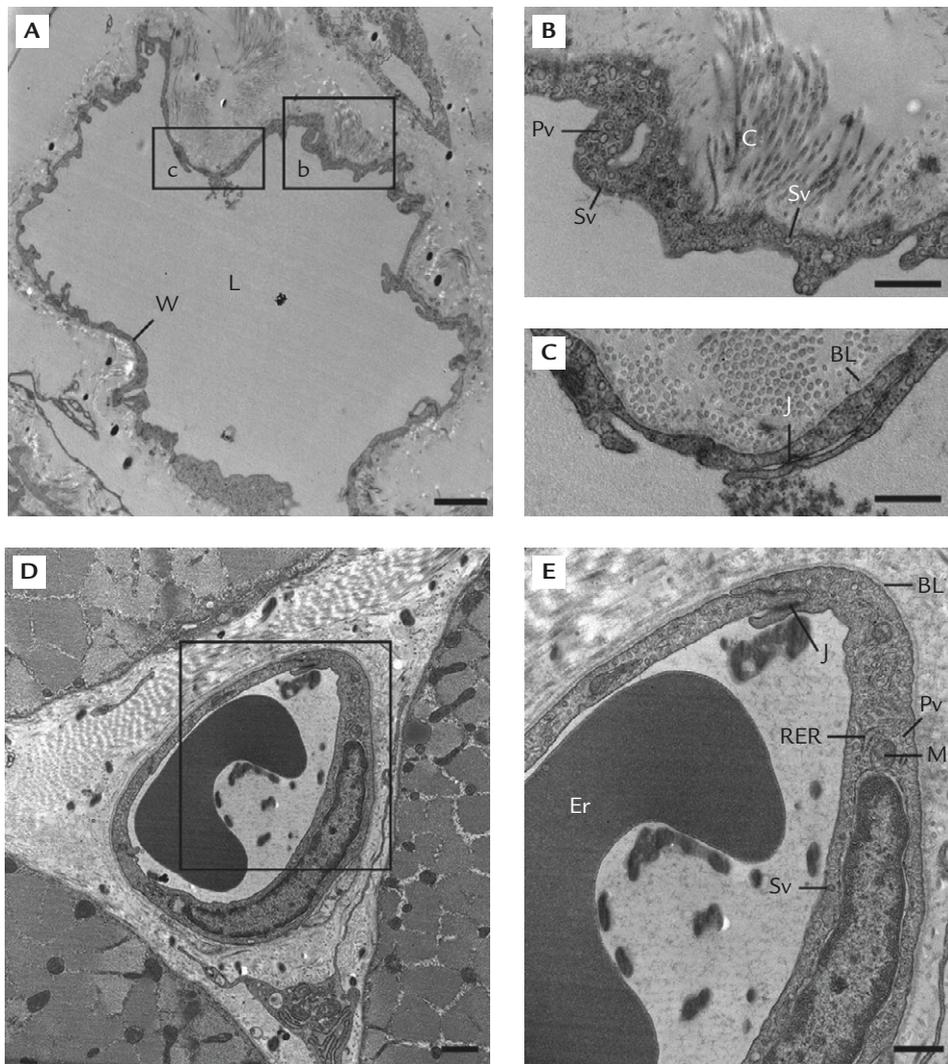


Figure 6 Electron micrographs showing ultrastructural characteristics of lymphatic (A) and blood (D) capillaries of rabbit. Scale bar, 2 and 1 μm , respectively. (B, C) magnified images of rectangular areas in Figure 6A; scale bar, 0.5 μm . (E) Magnified image of rectangular areas in Figure 6D showing blood capillary ultrastructural features; L=lumen; W=wall of lymphatic capillary; C=collagen fibers; Pv=pinocytotic vesicles; Sv=surface vesicles; BL=basal lamina; N=nucleus; M=mitochondria; RER=rough endoplasmic reticulum; J=junction of the endothelial cell; Er=erythrocyte; scale bar, 0.5 μm .

collapse [36]. Thus the absence of such anchoring filaments in Bonghan ductules presumably means that their function does not critically depend on their connections with ECM components. Third, although the cytoplasmic processes of DEC's formed overlapping and interdigitated interconnections, the cell membranes of DEC's did not show characteristic intercellular adherens junctions, which suggested that DEC's had weak or loose overlapping and interdigitated cell membranes that may open under the pressure of circulating liquid and may pass large macromolecules, such as hyaluronic acid and albumin, which were detected in significant amounts in rabbit BHCs by mass spectroscopy [26]. Indeed, when a staining solution was injected into a ductule,

its diameter increased [1], which could be attributed to weak intercellular junctions and the absence of basal lamina in the ductule. Fourth, the cytoplasm of ductule endothelial cells contained a well-developed granular endoplasmic reticulum and many free ribosomes and polysomes, an ultrastructural feature indicating that endothelial cells may actively synthesize and produce different peptides and glycoproteins, as well as glycosaminoglycans, abundant in Bonghan liquid [1]. Another distinct and notable morphological feature of the Bonghan ductules was the scarcity of pinocytotic vesicles and specific organelles such as Weibel-Palade bodies. These organelles are usually present in blood vessels and capillaries and contain different substances

Table Comparison of the morphological features of endothelial cells in Bonghan ductules and in blood and lymphatic capillaries

| Characteristic | DECs | BECs | LECs |
|--|---|---|---|
| Diameter of lumen | 6 μm minimum | 5 μm minimum | 20 μm minimum |
| Morphology of lumen | Small, regular | Small, regular | Wide, irregular |
| Majority of cells in lumen | Immune cells, small basophilic granules | Erythrocytes, leucocytes | Leucocytes |
| Type of endothelium | Single layer, nonfenestrated | Single layer, nonfenestrated | Single layer, nonfenestrated, extremely attenuated |
| Thickness range of endothelial wall | 0.2–0.7 μm | 0.2–0.7 μm | 0.2–0.4 μm |
| Presence of a basement membrane | No | Yes, continuous | Yes, discontinuous |
| Accessory cells | No | Pericytes/smooth muscle cells | No |
| Junction types | Overlapping and interdigitated | Interdigitated junctions adherens/tight/gap junctions | Overlapping and interdigitated junctions adherens/tight/gap junctions |
| Presence of gap junctions | No adherens/gap junctions | Adherens/gap junctions | Adherens/gap junctions |
| Presence of Weibel-Palade bodies | Lacking | Yes | Lacking |
| Amount of pinocytotic vesicles | Little | Many | Many |
| Amount of ribosomes | Many | Middle | Many |
| Level of development of granular endoplasmic reticulum | Well-developed | Poorly developed | Poorly developed |
| LYVE-1 receptor | No | No | Yes |

DEC = ductule endothelial cell; BEC = blood vessel endothelial cell; LEC = lymphatic endothelial cell.

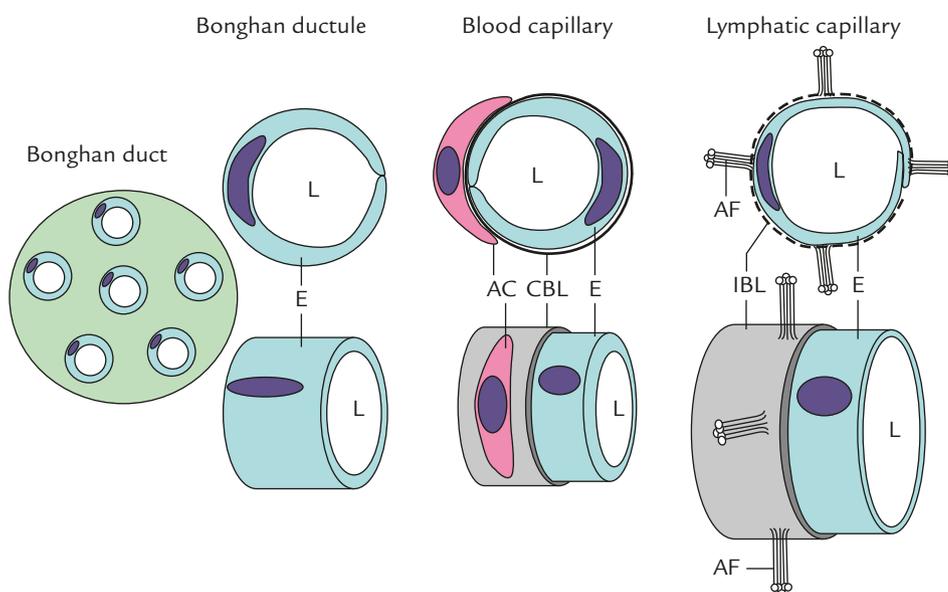


Figure 7 Structural properties of Bonghan ductule and of blood and lymphatic capillary. E=endothelium; L=lumen; AC=accessory cell; CBL=complete basal lamina; IBL=incomplete basal lamina.

for blood coagulation and inflammation, such as coagulating factor, “von Willebrand factor”, P-selectin, tissue plasminogen activator, endothelin-1, histamine, and different inflammatory chemokines [37]. The absence of Weibel-Palade bodies in ductule endothelial cell cytoplasm indicated that ductule endothelium was not involved in blood coagulation hemostasis.

Thus, due to distinct structural characteristics of Bonghan ductule endothelial cells described here, it may be concluded that these cells may express specific proteins that could be used as biomarkers to differentiate them from endothelial cells of blood and lymphatic vessels and capillaries. For example, lymphatic vessel endothelial receptor 1 (LYVE-1) was developed as a biomarker for lymphatic endothelial cells. In the future, identification of specific markers for ductule endothelial cells will create opportunities for new discoveries concerning the biological and physiological functions of the BH system.

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