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Various stem cells in acupuncture meridians and points and their putative roles

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ABSTRACT

Traditional Chinese and Korean medicine uses various manipulations on acupuncture points/acupoints that are located along imaginary lines on the surface of a human body, which are called 'meridians'. Acupuncture has been used from the ancient time till now to cure various diseases, including for the purpose of regenerative medicine. In various studies, meridians are alternatively called as Bong-Han ducts, primo vessels, or hyaluronic-acid rich ducts, while acupoints are called Bong-Han corpuscles, primo nodes, or hyaluronic-acid rich nodes. Meridians and acupuncture points form a system that is now called primo vascular system (PVS), which is claimed to contain various kinds of stem cells. The stem cell size is between 1-5 microns. The smallest is the primo microcells that have a putative role in regeneration. Other stem cells are adult pluripotent and hematopoietic stem cells that play a role in extra bone marrow hematopoiesis. The presence of PVS has been reproduced by many studies. However, the various stem cells need further studies to prove their existence and function, and harvesting PVS to isolate the stem cells might harm the health of the donor.

1. Introduction

Traditional Chinese and Korean medicine uses various manipulations on acupuncture points/acupoints that are located along imaginary lines on the surface of a human body, which are called 'meridians'. Acupuncture has been used from the ancient time till now to cure various diseases, including for the purpose of regenerative medicine. It is believed that there is a special structure under an acupoint, which responds to the manipulations that induces healing. Various findings showed that acupoints and meridians had special physical characteristics and structures that corresponded to interstitial connective tissue.[1–4] A study revealed that large intestine, liver and bladder meridians had lower electrical impedance due to the presence of subcutaneous collagen fibers, which could be visualized by ultrasound imaging as echogenic bands.[1] Another study showed the same phenomenon on pericardium and spleen meridians.[2] Moreover, a study on twelve subsets of pain trigger points showed that the resulting referred pain distributions were in accordance with their corresponding acupuncture meridians.[5] Yoga is believed to exert beneficial effects on various disease symptoms, and a study showed that the health benefits were due to improvement in acumeridian energy balance, which was assessed by using electrodermal measures.[6] However, a systematic review on accupoints and meridians concluded that the presence of acupoints and meridians was not conclusively proven by electrically distinguishable areas.[7]

A Korean scientist, Bong-Han Kim, was the first to discover special structures, which were related to acupoints and meridians. He named the structures under the acupoints as Bong-Han corpuscles, while the meridians were named as Bong-Han ducts, and both were parts of a Kyungrak (Bong-Han) system.[4,8–10] There are various Bong-Han systems (BHSs) according to the locations, namely superficial and deep BHSs.[9] The Bong-Han corpuscles of superficial BHS can be found under the acupoint on the skin, while deep BHS can be found in various locations, namely intravascular, extra vascular, organ surface, intra organ, and nervous BHSs. The intravascular BHSs are found inside blood and lymphatic vessels and in the heart, while extravascular BHSs are located outside and run along blood and lymphatic vessels and nerves. Organ surface BHSs are found on the surfaces and freely floating on visceral organs, while intra organ BHSs are located inside visceral organs. Nervous BHSs are found floating in cerebrospinal fluid in central

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and peripheral nerve system.[10] These findings were difficult to reproduce as the staining methods to show both Bong-Han corpuscles and ducts were not clearly described. Moreover, at about 1965, the institution where Bong-Han Kim worked was closed and his where about is unknown, which caused his findings to be forgotten for a long time.[9] Therefore, other scientists, who tried to reproduce his findings, were only partly successful.[4,10]

Around 40 years later, a team of Korean scientists re-investigated the existence of Bong-Han corpuscles and ducts, and found that they were indeed present.[11,12] In 2002, Kwang-Sup Soh, a member of the team coined the name of primo vascular system (PVS) that consisted of primo nodes and vessels. The primo vascular system, primo nodes and vessels correspond to the Bong-Han system, Bong-Han corpuscles, and ducts, respectively.[9] In primo vascular system, various kinds of cells can be found, including very small stem-like cells,[13] which express pluripotency marker Oct-4, and Nanog.[14]

Therefore, this review addressed the various terms in acupuncture and PVS, methods to visualize the PVS, including primo nodes and vessels, macroscopic and microscopic appearance of the nodes and vessels, stem cells of the PVS, and their putative roles in physiological and pathological conditions.

2. Various terms in acupuncture and primo vascular system

The meridians and acupuncture points/acupoints were described approximately 2,000 years ago in an ancient medical text that is known as 'Huangdi Neijing' (Yellow Emperor's Classic on Internal Medicine).[15] In the meridians flows the 'Qi' that is believed to be a live-giving fluid,[15] which was described as Bong-Han liquor by Kim,[9] and as primo vascular fluid or primo fluid (P-fluid) by Soh.[16] According to Bong-Han Kim, Bong-Han system contains granules, or some sort of small cells that are called 'sanals', Bong Han microcells, or primo microcells.[4,16,17] The terms concerning various structures, which are related to acupuncture meridians and point in medical literature according to various scientists, are shown in Table 1.

Primo fluid flows with a speed range of 0.1–0.8 mm/s, and is supposed to function as an optical channel for biophoton emission that might generate electromagnetic signal, which plays a role in various biological processes.[8]

The term primo vascular refers to the fact that PVS develops in vitelline membrane in eggs within 16–24 h after incubation, before the development of extra and intra embryonic vessels. It is suggested that the PVS is a primordial circulatory system that serves as a blueprint for the development of blood vessels.[8]

3. Methods to visualize the primo vascular system

Bong-Han Kim studies were not published in International journals and were written in Korean, except one study that was translated into English. Moreover, the methods were not explained in detail, except the use of 'blue dye' to visualize Kim's Kyungrak system (PVS).[10] The early attempts to reproduce the PVS study were done by groups of German, Japanese and Chinese scientists. However, the German study was unsuccessful due to the method, which was histological study, without first isolating the PVS. The Japanese study was published in a local journal in Japanese, while the other from China was unpublished, so soon the results were forgotten.[19]

Later on, a follow up study of the Japanese study was published in a book, which described that their method did not use any dye to visualize the PVS. Instead, they relied on gross anatomy to find and isolate the PVS on organ surfaces using a loupe and a stereomicroscope. After isolation, they made smashed tissue, spreading or paraffin sectioned samples to be stained using various histological staining methods. By this method they were able to show the superficial primo nodes on skin membrane, and deep PVS on organ surfaces, which partially correspond to the Bong-Han system.[20] In addition, a historical review of the study in china was published, which did not explain clearly the methods, except that they did perfusion to show PVS in blood vessels, and got no result. Furthermore, from the only picture showing a superficial Bong-Han corpuscle-like structure in young rabbit umbilical cord can be concluded that they used paraffin sectioned samples, which were stained by routine histological staining method.[21]

3.1. Various methods to isolate primo vascular system

Other attempts that clearly described the methods to isolate the PVS were a method using dextrose without using dye,[22] or contrast enhancing optical method,[23] followed by the use of various kinds of dyes and staining methods, including acridine orange,[24] combination of Mayer's hematoxylin and Feulgen,[12] alcian blue,[18] tryphan blue,[25] and Janus green.[26]

Methods to visualize the PVS are important as they make the isolation easier, and isolation is important for further studies. Successful isolation and microscopic descriptive studies on PVS mostly involved deep PVS,[12,18,22–26] while for superficial PVS, especially for primo nodes, only a part of Bong-Han Kim study was reproducible.[21,27]

Without staining, PVS resemble fibrin threads and clots, and therefore can be distinguished from fibrin threads and clots by using heparin containing solution to perfuse the blood vessels or blood covered organ surfaces to dissolve the fibrin.[21] For intravascular PVS, which is found in lymphatic vessel, the transparent wall

Table 1
Various terms in relation with meridians and acupoints.

Traditional name	Bong-Han Kim group[9]	Kwang-Sup Soh group[4,16]	Others[18]
Kyungrak[a] system	Bong-Han system	Primo vascular system (PVS)	hyaluronic-acid rich node and duct system (HAR-NDS)
Meridian = Jingluo (in Chinese)[15] Kyungrak (in Korean)[9]	Bong-Han duct	Primo vessel	hyaluronic-acid rich duct (HAR-D)
Acupuncture point (acupoint) = Xuéwèi (in Chinese),[15] Kyunghyul (in Korean)[9]	Bong-Han corpuscle	Primo node	hyaluronic-acid rich node (HAR-N)
Qi[15]	Bong-Han liquor	Primo vascular fluid, primo fluid, P-fluid	
	'Sanal'[b]	Bong Han microcell, Primo microcell, P-cell	

[a] Kyungrak = meridians and collaterals.
[b] 'Sanal' = 'live egg' (in Korean).[10]

of the lymphatic vessel may pose a problem. Therefore staining of PVS is usually a prerequisite to visualize and make the isolation of PVS easier.[12,18,24–26]

4. Macroscopic and microscopic appearance of primo vascular system

4.1. Macroscopic appearance of primo vascular system

Macroscopically, both superficial and deep PVS is composed of two main components, primo vessels and primo nodes. Primo nodes are interconnected by primo vessels. At both end of a primo node, branching and merging of primo vessels may be observed. The primo vessels and primo nodes are difficult to visualize, as they are very tiny. In general, a primo vessel looks semi transparent,[9,28] while a deep primo node is an enlargement of a deep primo vessel, with a dimension of 3–7 mm in length and 0.5–1 mm in width.[9] Another study showed that primo nodes from rat organ surfaces were 0.1–0.5 mm in width and 0.5–1 mm in length.[29] A deep primo node from rabbit is elongated or oval in shape, and has a milky appearance.[9,19,28]

Appearance of PVS is better revealed after isolation, and as the PVS is very tiny, an optical imaging system and skill in microsurgery is needed to isolate the PVS.[19] A primo vessel inside a rabbit's lymphatic vessel has a diameter of 20–30 μm,[29] and from rat's organ surface is 40–100 μm.[30] However, when it is filled by dye solution, the diameter becomes larger.[9] In a study, Alcian blue injection showed a primo vessel diameter of 50 μm,[29] while another study using Janus green showed a diameter between 26 – 500 μm.[26] Primo vessels contain circulating nucleic acid-rich fluid that is called the primo fluid. The nucleic acid mostly consists of deoxyribonucleic acid (DNA),[9] which causes positive staining of the primo vessels by Feulgen,[12] and acridine orange.[24] In addition, primo fluid is rich in hyaluronic acid that causes Alcian blue positive staining.[18]

4.2. Microscopic appearance of primo vascular system

Microscopically, a primo vessel may contain 1-20 ductules,[30] which are alternatively called as primo lumens.[8] A small ductule has a lumen of 6–10 μm,[9,28] while a larger one has a diameter of 30–50 μm.[9] In general the ductule is lined by a sort of non fenestrated endothelial cells without adhering or gap junction and basement membrane. The endothelial cells have rod-shaped nuclei of 10–20 μm in length,[9,28] and are Von Willebrand factor -positive, but CD31-negative.[31] However, a double staining study showed that the ductule had a double membrane, which was composed of the endothelial cells as the inner membrane, and epithelial cells as the outer membrane.[19,31] Fibrin-like fibers and amorphous intercellular substance is found in between the ductules (Fig. 1). The ductules contain basophilic granules, and nucleus-like bodies.[19,28] The basophilic granules are DNA granules that were regarded as extracellular DNA microvesicles (eDNA),[8] or regarded as very small cells that are called 'Sanals' (primo microcells).[17,32]

Microscopically, in general, a deep primo node is covered by a thin membrane that is composed of inner endothelial cells and outer epithelial cells,[31] and contains various kinds of cells, namely mast cells, histiocytes, and various blood cells, including granulocytes (neutrophils and eosinophils), lymphocytes,[28,31] and monocytes, which all are embedded in intercellular substance.[28] In addition, round immature cells that are possibly hematopoietic stem cells, a lot of chromaffin cells,[31] and very small embryonic stem-like cells (primo microcells) are present.[9,19] The cells are scattered, or forming clusters, which contain or are located near small channels or ductules with a diameter of 7–15 μm, and the lumen is lined by one layer of endothelial cells. The ductules in deep primo node may contain basophilic granules of up to 1 μm.[28]

5. Stem cells of the PVS, and their putative roles in physiological and pathological conditions

Primo vascular system contains various kinds of stem cells, including primo microcell, putative hematopoietic stem cells, and cancer stem cells in cancer patients.[9,13,18,19,31,33–37]

5.1. Primo microcell

Several studies showed that primo microcell expressed pluripotent stem cell markers, and their size ranged from one to four μm.[4,14,33] In one of the studies, CD133 positive cells were regarded as primo microcells, and selection of CD133 cells using CD 133-magnetic beads was conducted. The primo microcell was around 3–4 μm in size, had a round, dark, eccentrically located nucleus without nucleolus, and had a medium nuclear to cytoplasmic ratio. The nucleus was positive for Oct4 and Nanog in immunocytochemistry staining. The cytoplasm formed bud-shaped protrusions and contained a lot of basophilic dots that might be ribosomes. However, the number of cells after selection was very low, only 0.05% of total nucleated cells, and the figure of Oct4 and Nanog staining showed only one positive nucleus and invisible cytoplasm in the whole field.[14]

The other study found that the size of primo microcell was smaller, i.e. 1–2 μm, and conducted reverse transcription polymerase chain reaction (RT-PCR) to study the expression of various pluripotent stem cell markers on whole extract of primo vessels. The results showed that all samples were positive for Oct4, two of three samples were positive for Sox2, Stella, Rex1, Klf4, one sample was positive for cMyc, and none was positive for Nanog.[33] As the RT-PCR in this study was done on whole extract, the pluripotent markers did not specifically belong to primo microcells, thus other types of pluripotent stem cells might be present as well.

Primo microcells are believed to migrate through PVS to accumulate in damaged tissues/organs, and exert regeneration of the tissues/organs, which is a well known function of tissue/organ's resident stem cells. Moreover, primo microcells were observed to exert Brownian motion, and their speed of motion was significantly increased by UV light of 360 nm (UV-A), but not altered by visible light; therefore, the physiological meaning of this fact needs further studies.[4]

Regarding the size, primo microcells might be very small embryonic like (VSEL) stem cells that first were isolated from bone marrow and described by Ratajczak et al.[38,39] The presence of VSEL stem cells was questioned, as many other researchers could not reproduce the finding,[40,41] though a study succeeded to isolate mouse bone marrow VSEL and propagate them using C_2C_{12} feeder.[34] Primo microcells contain fragmented DNA,[4,13] and their chromosomes were incomplete both in number and structure.[4,13,35,36] Therefore, small primo microcell could be apoptotic bodies that ranged from 0.5 to 2 μm,[35,42] or large microvesicles[33] that might have a size of 1 μm,[42] and could not be regarded as cells.

5.2. Other types of stem cells in primo vascular system

Several studies showed the presence of other stem cells besides primo microcell in PVS. A study showed that PVS contained adult pluripotent stem cells and hematopoietic stem cells. The adult pluripotent stem cells could differentiate into $CS45^{-}Flk1^{+}$ hemangioblast-like cells when cultured using OP9 cells as feeder layer, while the hematopoietic stem cells could form erythroid, granulocyte-macrophage, and mast cell progenitors when cultured

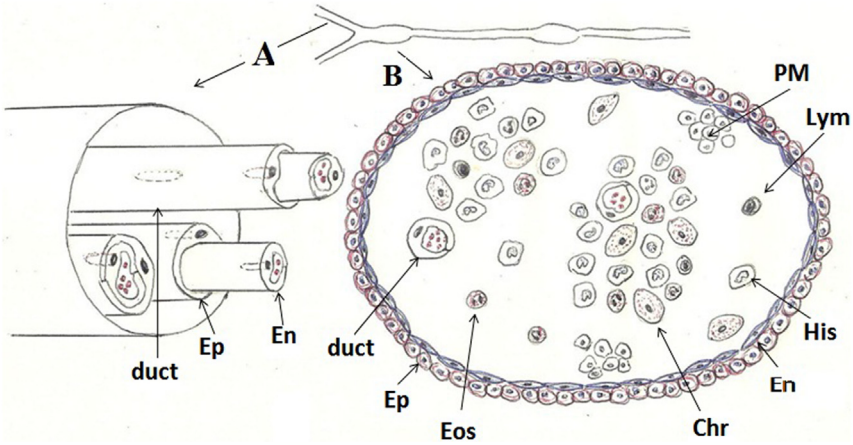

Fig. 1. Schematic representation of a primo vessel. A = primo vessel, B = primo node, duct = ductule, Ep = epithelial cell, En = endothelial cell, Eos = eosinophil, Chr = chromaffin cell, His = histiocyte, Lym = lymphocyte, PM = primo microcells.

in methylcellulose-containing special medium to assess the various colony forming units. Green fluorescent protein (GFP) tracking of PVS and bone marrow derived stem cells, which were given to lethally irradiated B6 mice showed that PVS stem cells could not engraft, while bone marrow derived stem cells could and were found to engraft in PVS.[18]

The presence of adult pluripotent and hematopoietic stem cells in PVS[18] suggests hematopoietic role of intravascular PVS. This suggestion is in line with the findings of enlarged primo vessels that make them easy to detect, and the presence of immature red blood cells in organ surface primo nodes in phenylhydrazine induced anemia.[17]

Another study isolated 2–5 μm Sca-1 $^{+}$ Lin $^{-}$ CD45 $^{-}$ stem cells from mouse PVS. The size of 2–5 μm, and Sca-1^{+} Lin $^{-}$ CD45^{-} are the properties of VSEL stem cells. The study found that the frequency of those cells was up to 2.32 %, a 100 fold higher compared to VSEL stem cell frequency from mouse bone marrow, which was only 0.02 %. Further, PVS stem cells were positive for Oct4, Sox2, SSEA-1, and Nanog, while bone marrow VSEL stem cell was only positive for Oct4, Nanog, and SSEA-1. PVS stem cells were negative for apoptotic markers 7AAD and annexin V, and when cultured on C_2C_{12} feeder, they could proliferate and form spheres. PVS stem cells could produce up to 176 spheres per 1000 cells, while bone marrow VSEL stem cells only formed up to 14 spheres per 1000 cells, and repeated cultures increased the number of PVS stem cell spheres, but not the number of VSEL stem cell spheres that remained constant. Upon neuronal induction, both PVS and VSEL stem cells could differentiate into NeuN- and MAP-2-positive neurons, and when labeled PVS stem cells were injected intravenously to mice with ischemic brain injury, the infarct size was decrease, and labeled NeuN positive stem cells could be found in the ischemic brain.[34] This study suggests that PVS stem cells are promising to be used in cell therapy, but harvesting PVS to isolate the stem cells will damage the PVS, and may cause harm to the donor.

5.3. Cancer stem cells in primo vascular system

Animal studies on human or murine derived metastatic tumors suggested that tumor cells might use the PVS as a means of invasion to remote places,[37,43–46] and development of new PVS around the tumor preceded neovascularization.[47] Further, a study showed that human tumor derived stem cells, which were KLF4 positive, were found in the PVS; and KLF4 is a marker of pluripotent stem cells.[37] Therefore, it was suggested that PVS might provide a niche for cancer stem cells.[37]

5.4. Development of microcells

Microcells are naturally found in tumor tissue; they are metabolically active, and some researchers believe that they derive from cells undergoing apoptotic cell death. However, development of microcells is different from apoptosis, and Buikis coined the term 'sporosis' as the process of developing microcells (Fig. 2).[4,48] Technologies to produce artificial microcells were established, and these microcells were used to transfer a whole chromosome or chromosome fragment into recipient cells by fusion with the recipient cells.[49] Whether this mechanism of material transfer occurs in natural physiologic condition, and whether transfer of needed materials to degenerating cells might serve as a repair mechanism to regenerate a damaged tissue need further studies.

5.5. Proliferation and generation of primo microcell

The small primo microcells were described to proliferate in culture,[36] which then could fuse to form a new cell,[32] or a bigger cell-like body without a perfect nucleus.[36] This phenomenon is believed to be the origin of the various stem cells in the PVS, which

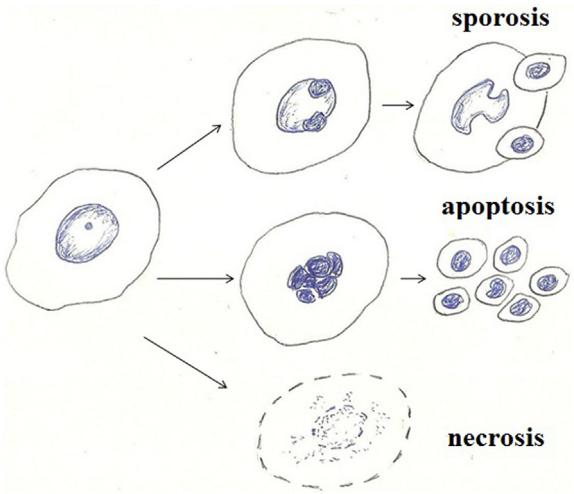

Fig. 2. Development of microcells ('sporosis').

might serve in regeneration of damage cells in the PVS surroundings.[36] However, the observation of fusion of some primo microcells to form a bigger cell might be an artifact, and therefore need further studies to prove it, whether such fusion could give rise to functional stem cells.

Moreover, primo microcell might be generated from existing cells in the vicinity,[32] which experienced apoptotic cell death that evaded phagocytosis by macrophages,[35] thus small primo microcells might be apoptotic bodies, and not functional cells. However, a study showed that they were different from apoptotic bodies,[13] and hence primo microcells might have a certain function that was not elucidated yet.

Baik et al, 2009 described the proliferation of primo/Bong-Han microcell (BH-MC) as: "Initially the BH-MC protrudes a thread and produces a daughter microcell from that thread, such that with proliferation, it forms a bundle of BH-MCs, which then fuse to make a nucleus-like structure. Finally, the structure is enclosed by a membrane to form a cell. About 10% of the BH-MCs observed in SEM images showed such protrusions, having a threadlike structure 100 nm in diameter and 400 nm in length".[13] Another study also observed budding and formation of thread-like structures between two cells in primo microcell culture.[33] However, such budding does not correspond to commonly known human cell proliferation process, and correspond more to proliferation of yeast.[50] Instead, a threadlike protrusion is more similar to a filopodia, which in human serves in cell movement, neuronal growth-cone formation, and invasion,[51,52] while budding is more similar to pseudopodia, which is involved in cell movement, and thread-like structures between two cells may by a tunneling nanotube for cell-cell communication and trafficking of cell components between cells.[53] Therefore, more studies are needed to prove the proliferation of primo microcells, and whether they undergo the commonly known mitosis or other mode of proliferation.

6. Conclusion

Meridians and acupuncture points form a system that is now called primo vascular system (PVS), which is claimed to contain various kinds of stem cells. The stem cell size is between 1-5 microns. The smallest is the primo microcells that have a putative role in regeneration. Other stem cells are adult pluripotent and hematopoietic stem cells that play a role in extra bone marrow hematopoiesis. The presence of PVS has been reproduced by many studies. However, the various stem cells need further studies to prove their existence and function, and harvesting PVS to isolate the stem cells might harm the health of the donor.

Conflict of interest statement

The author declares that she has no conflict of interest.

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