Damianus *Journal of Medicine*
Vol.24 No.1 April 2025: hal.78-89

ARTIKEL TINJAUAN PUSTAKA

STRUCTURE, MORPHOLOGY, AND ROLE OF PRIMO VASCULAR SYSTEM

STRUKTUR, MORFOLOGI, DAN PERAN SISTEM VASKULAR PRIMO

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ABSTRAK
Pendahuluan: Kim Bong Han pada tahun 1960 memperkenalkan sistem primo vaskular (PVS), sebuah sistem peredaran baru yang tidak bergantung pada sistem peredaran darah dan limfatik. Sistem ini diyakini mempunyai hubungan dengan titik-titik akupunktur klasik dan meridian yang mendasari mekanisme akupunktur dalam penyembuhan penyakit. Review ini bertujuan untuk lebih memahami struktur, morfologi dan peran sistem pembuluh darah primo.
Metode: Data dikumpulkan dari jurnal internasional menggunakan kata kunci terkait Primo Vascular System dari Google Scholar, Proquest, Scopus, dan Pubmed. Dari hasil penelusuran diperoleh 30.336 artikel dan disaring sesuai kriteria inklusi dan eksklusi. Ekstraksi data mencakup *original articles* berbahasa Inggris yang diterbitkan antara 2019-2022, *open access*, dan *free full text*.
Hasil: Dua belas penelitian relevan dianalisis dan ditemukan bahwa sistem pembuluh primo memiliki struktur dan bentuk yang berbeda dari sistem vaskular dan limfatik, PVS berupa bintik-bintik semi transparan berwarna putih susu dengan pembuluh primo dan kelenjar primo di dalamnya terdapat cairan primo yang bersirkulasi dan mengandung berbagai sel, termasuk sel mikro primo yang mengandung DNA. Sistem Primo Vaskular dapat berperan dalam regenerasi jaringan, imunitas bawaan dalam proses inflamasi, dan hematopoiesis ekstrameduler.
Simpulan: Sistem primo vaskular terdiri dari bintik-bintik putih susu, semi transparan dengan pembuluh darah primo dan kelenjar getah bening, berperan penting dalam regenerasi jaringan, imunitas bawaan, dan hematopoiesis ekstrameduler.
Kata Kunci: sistem Bong Han, sistem Kyung-Rak, primo-microcells, sistem primo vaskular

ABSTRACT
Introduction: *Kim Bong Han in 1960 introduced the primo vascular system (PVS), a novel circulatory system independent of the circulatory and lymphatic systems. This system is believed to have a relationship with classical acupuncture points and meridians which underlie the mechanism of acupuncture in healing diseases. This review aims to understand better the structure, morphology, and role of the primo vascular system.*
Methods: *Data was collected from international journals using keywords related to Primo Vascular System, from international journals, including Google Scholar, Proquest, Scopus, and Pubmed. From the search results, 30,336 articles were obtained and and filtered according to the inclusion and exclusion criteria. Data extraction includes original English articles published between 2019-2022, open access, and free full text.*
Results: *Twelve relevant studies were analyzed and it was found that primo vascular system has a different structure and shape from the vascular and lymphatic systems, the PVS is milky-white, semi-transparent spots with primo vessels and primo nodes inside of which primo fluid circulates and contains a variety of cells, including primo microcells that contain DNA. The Primo Vascular System can play a role in tissue regeneration, innate immunity in inflammatory processes, and extramedullary hematopoiesis.*
Conclusion: *The primo vascular system, consisting of milky-white, semi-transparent spots with primo vessels*

and nodes, plays a crucial role in tissue regeneration, innate immunity, and extramedullary hematopoiesis.
Key Words: *Bong Han system, Kyung-Rak system, primo-microcells, primo vascular system*

INTRODUCTION

Acupuncture is a medical practice in China, Japan, and Korea where the mechanisms underlying it are still not clear. The Primo Vascular System is a system of anatomical features that correspond to traditional acupuncture sites and meridians, according to numerous research. This system was discovered by Kim Bong Han in 1960 who was introduced as the Bong Han system.[1] This system has special structures and characteristics that can respond to the manipulation of acupuncture points so that they can cure various diseases.[2] Kim Bong Han describes this system as a node and channel that are interconnected with the acupuncture points in the ancient acupuncture meridians.[3]

Kim Bong Han received his medical degree from Seoul National University's College of Medicine in 1941. From 1962 to 1965, Kim worked as the director of North Korea's Kyung-Rak Research Institute. Kim's research was supported by the Government of North Korea by supplying various research instruments such as radioactive tracers and microscopes.[4] Kim published his research in five articles on (1) the body parts that correlate to meridians and acupuncture points, (2) the morphology and function of the Kyung-Rak system, (3) systematic about the Kyung-Rak system, (4) Sanal cells or primo microcells, and (5) Sanalization and hemato-poiesis.[5]

Following the inaugural International Symposium on the Primo Vascular System in 2010, the Kyung-Rak system (Bong Han system) was referred to as the Primo Vascular System. Primo nodes, Primo vessels, Primo sub-vessels, Primo fluid, and Primo microcells make up the Primo Vascular System. The word primo refers to an early developmental stage before the development of blood vessels or nerves that are important in organ regeneration and health. Kim classified the PVS into superficial PVS, internal PVS, intra-external PVS, external PVS, and intra-organic PVS.[6]

Based on the above background, it is found that not much is known about the primo vascular system, the authors are interested in exploring the structure, morphology, and role of the primo vascular system.

METHOD

Search for data sources in this review through international journal pages, namely Google Scholar, Proquest, Scopus, and Pubmed. Search for journals and articles using key words in English, namely "Primo Vascular System". From the search results, 30,336 articles were obtained and filtered according to the inclusion and exclusion criteria to be analyzed with appropriate topics. Data extraction includes the year of publication, open-access journal, methods, and results obtained.

The inclusion criteria for this study include original articles or research articles in English, scientific articles published from 2019 to 2022, open access, and free full text. Exclusion criteria in this study include review articles, journals that are not following the timeframe (2019-2022), journals that are not in English, not open access, paid journals, and those that are not following the topic of study.

RESULT

Based on the result of the journal search as previously mentioned, 12 journals were obtained which will be explained in Table 1.

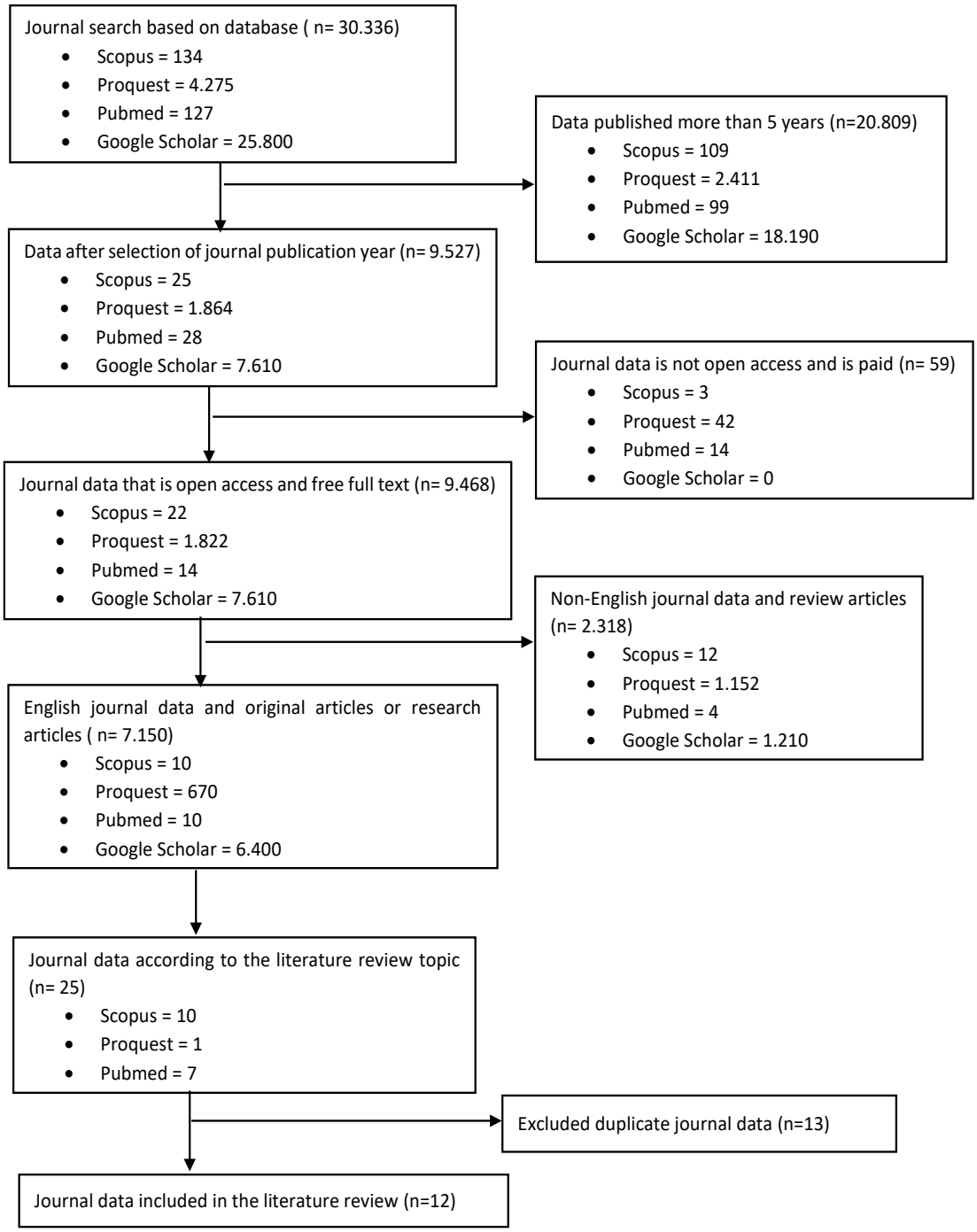


Figure 1. Flowchart of Literature Review

Table 1. Primo Vascular System

Research	Objectives Research	Methods Research	Results
Scholkman F, Shen Y, Ryu PD. 2019.[7]	To investigate the anatomy and function of the PVS through experimental research	Male Sprague Dawley rats aged 5-7 weeks were anesthetized with alfaxalone and xylazine, then surgically opened to search for thread-like structures, and analyzed using phase contrast microscopy.	PVS was discovered in 5 rats, exhibiting a semi-transparent, milky white, mottled structure with red sections, possibly from erythrocytes, suggesting extramedullary hematopoiesis.
Mustafa FEA, 2019.[8]	To detect PVS in rabbit placenta	3 adult New Zealand white rabbits, 4-5 months old, female mated with male rabbits of the same species. Immediately upon slaughter, the entire uterus is repaired. Then the placenta was analyzed histologically.	The placenta's maternal side has a PV with an average thickness of 52.21 ± 8.341 μm, surrounded by epithelial cells and a pigmented nucleus. It contains basophilic granules, granulocytes, and lymphocytes, but no erythrocytes.
Shin JY, Ji JO, Choi DW, Choi SH, Choi JG, Rho MS, et al. 2019.[9]	To investigate changes in target gene expression patterns following inflammation induction by LPS and acupuncture electric stimulation at ST36 and LI04.	New Zealand female rabbits were anesthetized, injected with LPS, and divided into two groups. PV was observed in lymph vessels, and RT-PCR was used to analyze inflammation-associated genes and lymphatic endothelial cell markers.	PVS found in lymph vessels, plays a crucial role in gene activation and inflammation, with increased expression of genes like Flt4, Lyve-1, Prox-1, and Pdpn after AES at two acupuncture points.
Shin JY, Choi SH, Choi DW, An YJ, Seo JH, Choi JG, et al. 2019.[10]	To know the basic nature of the gene from rabbit PV.	Thirty New Zealand rabbits were divided into three experimental groups, each with a different primo vessel group. Alcian Blue was injected into the nodes, and differentially expressed genes were analyzed.	RNA-seq results reveal 30 genes expressed differently in primo vessels, primo vessels + lymph vessels, and lymph vessels only. Primo vessels showed significantly different gene expression patterns for 10 genes, increasing fragments per kilobase of exon per million compared to lymph vessels.
Choi BK, Hwang SH, Kim YI, Singh R, Kwon BS, 2019.[11]	Analyzing the composition of NDS (Node and Duct System) when stable and active, production of cytokines and chemokines under inflammatory conditions, physiological and cellular changes in vivo after proinflammatory stimulation of NDS	The study involved male C57BL/6 mice aged 4-5 weeks and those with TLR2-/-, TLR4-/-, and RAG1-/- immune cell subsets. NDS was collected from the peritoneal cavity and analyzed using flow cytometry, following intraperitoneal injection of 1x107 MC37 cells, and stimulated with LPS/zymosan and cytokine detection.	The study found NDS in male C57BL/6 mice aged 4 weeks, with macrophages dominating nodes. Proinflammatory stimulation increased cytokine and chemokine expression, causing neutrophils and macrophages to migrate and differentiate, indicating that NDS is an immunological organ.
Lim CJ, Yoon YS, Ryu PD, 2020.[12]	The morphology of cells on the PVS surface was examined using immunohistochemistry, scanning electron microscopy, and H&E staining.	PVS was isolated from the surface of the abdominal organs of male Sprague Dawley rats, weighing 100-180 g which found semitransparent milky PVS tissue. Furthermore, tissue characterization of cellular morphology was carried out by HE staining and immunohistochemistry as well as SEM examination.	The study reveals a dark core with thin, linear, elliptical, and spherical nuclei at junctions, and mesothelial cells covering abdominal organ surfaces and walls. Immunostaining with HBME-1 antibody confirmed dense immunoreactivity and SEM observations revealed squamous mesothelial cells on the PVS tissue surface.
Lim CJ, Shen Y, Choi MC, Ryu PD, 2020.[13]	Micro-CT analysis and light and electron microscopy validation	PVS was isolated from 5-week-old male Sprague Dawley rats and hemolytic anemia model rats	Micro-CT analysis of PVS tissue revealed primo bundles with ductules in the PN structure, with

Research	Objectives Research	Methods Research	Results
	of the 3D structure of the PVS network.	and analyzed using a micro-CT scanner, SEM examination, and H&E staining.	no significant differences between anemia and control groups in PN and PV sinuses.
Zhang L, Oh SW. 2020.[14]	To make monoclonal antibodies from mice against PV and PN and analyze these antibodies properties using ELISA, Western blotting (WB), and immunofluorescence (IF) microscopy.	The study isolated PVS from organ surfaces in male Sprague Dawley rats and then injected them into mice as an immunogen. The monoclonal antibodies produced were analyzed using cell fusion techniques, hybridoma screening, ELISA, EB, and IF. Negative control was used to control the process.	Four monoclonal antibodies (α-rPVS-m1-1, α-rPVS-m3-2, α-rPVS-m3-4, and α-rPVS-m4-6) were characterized using ELISA, EB, and IF, showing strong affinity for PVS but no specificity on IF.
Shen Y, Kim YJ, Ryu PD, 2022.[15]	Investigating the role of adrenergic signaling in morphological changes in heart failure rats and hemolytic anemia rats.	The study used male Sprague Dawley rats aged 5 weeks as a model for heart failure and hemolytic anemia, comparing the effects of norepinephrine alone and with α- or β-adrenoceptor blockers, and performed hematological analysis and PVS isolation.	Heart failure and hemolytic anemia rats with PVS showed higher sample counts, larger PNs, and more red samples. Propranolol inhibited these morphological changes, while phentolamine slightly increased them. Adrenergic signaling controls hyperplastic changes in PVS on the organ surface.
Li T, Tang BQ, Zhang WB, Zhao M, Hu Q, Ahn A, 2021.[16]	Re-evaluate the phenomenon of tracer dye in the pericardial canal on the forearm of a healthy human.	The study involved 15 volunteers aged 30-56, identifying pericardium meridians and acupuncture points on the forearm. Fluorescein sodium and indocyanine green were injected intradermally and observed using a laser beam, ultrasound imaging, and infrared vein detector.	In 19 trials, fluorescein injected into PC6 diffused slowly along a pathway matching PC, resulting in a blended dye. ICG-injected PC5, PC6, and PC7 showed similar trajectories but differed in migrating proximally and failed to coalesce on PC3. Ultrasonography and vein detection showed no suitable blood vessels in the tracking line.
Vodyanoy V, Pustovyy O, Globa L, 2022.[17]	To obtain primo nodes from rat femurs, characterize the cellular composition of the nodes, and structure of the nodal vessels, and compare them with other primo nodes and vessels.	A total of 190 primo nodes were obtained from 42 adult male Sprague Dawley rats, which were then microdissected and divided into femur bone parts. Fluorescence microscopy and immunohistochemistry were used for analysis.	Primo nodes, located in the bone marrow, are surrounded by a fine capsule and have inlet and outlet vessels. They are found in megakaryocyte family cells, including extracellular matrix and stem cells. The bone marrow is an intra-organic primo vascular node suitable for regenerative therapy.
Choi SH, Choi JG, Lee SS, 2022.[18]	Observing the effect of Foralumab which is an anti-CD3 monoclonal antibody on primo vessels induced inflammation with lipopolysaccharide (LPS).	A 10-week-old New Zealand female rabbit was administered LPS, Alcian Blue, and monoclonal antibody to lymph nodes near the abdominal vena cava. Observations were made using TEM and EDS, revealing the presence of dye.	Primo vessels in rabbits with LPS-induced inflammation appeared clearer, darker, and thicker after Foralumab administration, possibly due to activation of T lymphocytes in the lymphatic system.

DISCUSSION

Based on the results of a review of 12 research articles on the primo vascular system, it shows that this system is a unique system besides the vascular system and lymphatic vessel system.[14,16] In 1960, Kim Bong Han asserted that there was a connection between the primo vascular

system and acupuncture meridians. This system consists of primo nodes (PN) which are connected via primo vessels (PV) with other PNs.[1,3,7,13] The location of PVS can be identified into several subtypes including (1) superficial PVS which is in the skin and often shown on the meridian map, (2) internal (intravascular) PVS that are found in the blood or lymphatic vessels and floats within them (Figure 1), (3) intra-external PVS that are found freely on the surface of organs are often called organosurface PVS (Figure 2), (4) external PVS those outside the blood vessels or independent lymphatic vessels and these subtypes connecting to superficial PVS, (5) neural PVS that pass through the spinal canal and are located in the peripheral or central nervous system, (6) intra-organic PVS that are in in organs and are usually smaller than the other subtypes ranging from 0.1 mm to 0.5 mm. Many studies have found PVS in various organs and tissues extracted from a variety of animals, including pigs, dogs, rats, rabbits, cows, and mice. PVS was identified as originating from various organs such as the brain, sciatic nerve, spinal system, heart, blood vessels, lymph vessels, abdomen, testes, skin, and vitalin membranes was also found in cancer.[19,21] In the 12 articles analyzed, PVS were isolated from several locations, including internal PVS, intra-external or organosurface PVS, one external PVS, one neural PVS journal, and one intra-organic PVS that came from rats, mice, rabbits, or people.[7-18]

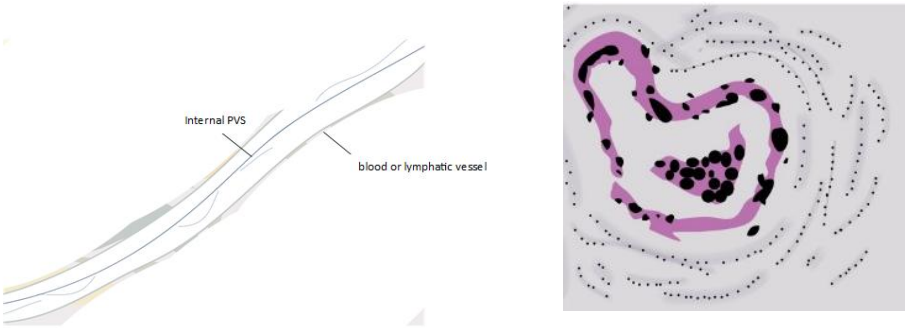


Figure 1. (a) Illustration of primo vessel in internal (intravascular) PVS colored with blue dye ; (b) cross section of a lymph vessel containing PVS.



Figure 2. Illustration organosurface PVS on the surface of intestine (a) and liver (b). PVS appears milky white, semi-transparent and consist of nodes and vessels.

This system is anatomically and histologically different in structure from the system of vessels and lymph vessels. Besides consisting of primo nodes and primo vessels, the primo vascular system has a fluid circulating within it which is referred to as primo fluid. Primo fluid has biochemical components including nucleic acid, ribonucleic acid, nitrogen, fat, reducing sugar, hyaluronic acid, 19 free amino acids, and 16 free mononucleotides. Within the primo vascular system, there are also primo microcells containing DNA which can act like embryonic stem cells.[5,6,19] Milky white, semi-transparent, and slightly flexible tissue consisting of nodes and vessels.[20] PVS without staining also looks like fibrin threads and clots, so to distinguish threads and fibrin clots you can use a solution containing heparin which can dissolve fibrin on the organ surface covered in blood. However, for primo vessels that are in blood vessels or lymph vessels, staining must be used to detect their presence.[2] Research by Scholkman F, Shen Y, and Ryu PD (2019) found PVS on the surface of the abdominal organs with a semi-transparent spot structure, milky white color , and there is one specific finding regarding the red section without magnification which consists of four primo nodes and one primo vessel. From these specific findings, it is possible that the red color is due to the presence of extramedullary hematopoiesis in the primo node and primo vessel which indicates the occurrence of erythropoiesis and reticulocytes were found in the PVS sample which strengthens the possibility of extramedullary hematopoiesis.[7] According to Hossein AM, et al (2012) on the surface of the pig's abdomen (between the liver and stomach, and between the small intestine and stomach) PVS is identified by a thread-like structure with a diameter of 100-300 μm and there is a thicker part like a capsule in between.[21]

In the research of Lim CJ, Yoon YS, and Ryu PD (2020) it was identified that PVS is covered by a layer of cells with a dark nuclear structure arranged on a thin nuclear basement membrane and arranged linearly (10-14 μm), elliptical (8-10 x 3-4 μm), and round (5-7 μm). These PVS-enveloping cells showed immunoreactivity with HBME-1 antibody which is a selective marker against mesothelial cells. The morphology of the mesothelial cells was identified using SEM with high magnification in the form of elliptical or halibut-shaped cells with larger sizes, having microvilli on their surface, as well as stomata in the extracellular matrix and boundaries between cells.[12] Mesothelial cells have various functions, including synthesizing and releasing hyaluronan, an immune response mediator that regulates inflammatory processes, immune responses, tissue repair, and protects tumor spread.[12] In primo vessels there is a primo lumen composed of 1-20 subvessels also called ducts or sinuses with a diameter of 3-25 μm. A duct's lumen measures 6 to 10 mm for small ducts and 30 to 50 mm for larger ones.[2,22] The primo subvessel is covered by a thin sheath of muscle-like endothelial cells with a diameter of 15-20 μm and a rod-shaped nucleus along

the subvessel axis.[22]

In PVS there are various cellular structures including endothelial cells, erythrocytes, chromaffin cells, immune cells, and primo microcells as illustrated in Figure 3.[23] Endothelial cells line the sinuses with rod-shaped nuclei arranged like dotted lines along the direction of the primo vessel. Endothelial cells are different from blood vessels and lymph vessels. These endothelial cells show negative expression for LYVE-1 and CD31 as markers of endothelial cells for lymph vessels and blood vessels.[23] In Lim CJ's study (2013) found erythrocytes in PVS with a biconcave structure measuring 6-8 μm, without nuclei and in the center paler in color. In addition, large round cells were found with a size of 12-20 μm, curved nuclei, and cytoplasm containing basophilic granules which were identified as mast cells. Multilobe identified as neutrophils, monocytes, and lymphocytes.[8,25] In Lim's research, biconcave cells without nuclei were also found which were identified as red blood cells or erythrocytes.[25] Choi BK's research (2019) found macrophages in PVS after being induced with LPS and zymosan.[11] In the primo node you can also find clusters of cells in the area near the sinuses with an oval-shaped cell structure and identified as chromaffin cells containing adrenaline and noradrenaline.[23] According to Kim Bong Han (1965) in the PVS there are cells with a spherical shape measuring 0.8-2.4 μm which are known as primo microcells or sanals.[5] Research by Ogay and Soh (2012) found high school small stem-like (SSL) stained with Hematoxylin-eosin measuring 3-4 μm, round nucleus shape, and basophilic cytoplasm believed to be primo microcell.[26]

Primo microcells are found in primo nodes in the abdominal cavities of rats and rabbits which play a role in wound healing and regeneration of damaged tissue.[19] By immunohistochemistry it was found that primo microcells express CD133 which is known as a stem cell marker for cancer and normal tissue. In addition, primo microcells also express Nanog and Oct-4 which are known as embryonic stem cell markers. Sanal cells, or primo microcells, serve as a stem cell niche and contribute to tissue regeneration.[26] Ahn SH, et al (2012) in their research found that primo microcells isolated from primo nodes experienced growth within 8-9 days of culture to or cell like bodies.[27] A sanalosome or primo microcell is composed of a sanalosome that contains large amounts of DNA and plasma channels surrounding a sanalosome which contains RNA. They have the same base and nucleotide composition as in normal cells.[5] Lee BC, et al (2012) found in PVS homogenates incubated in fertilized egg albumin with RPMI medium that primo microcells experienced bud growth which was observed to increase in size at 0, 21, and 52 minutes and showed a DNA signal from acridine orange as a DNA dye. The results of this study indicate that stem cells can emerge from primo microcells.[28]

PVS is known as the third circulatory system besides the circulatory system and lymphatic system, so it is necessary to

identify it in molecular biology through analysis of the gene expression of PVS. Shin JY, et al (2019) conducted a gene expression analysis in PVS rabbits which showed very different gene expression patterns for the 10 genes in group 1 (only primo vessel samples) vs group 3 (consisting of lymph vessels only) and FKPM, or fragments per kilobase of exon per million, increased two to three times in primo vessels compared to lymph vessels for 10 genes, including IGHM, HLA-DRA, HIST1H41, LPL, CD36, SRGN, DGAT2, SNCG, CD48, and GPD1.[10] In another study, Shin JY, et al (2019) found increased expression of the Flt4, Lyve-1, Prox-1, Pdpn genes in PV compared to lymphatic endothelium as well as increased Mtf2, Hif1a, Agtr1, Agtr2, Pparg after AES at two acupuncture points (ST36 and LI04) and lipopolysaccharide injection. These findings indicate the expression of lymphatic endothelial cells (LEC) marker genes in PVS whose expression can be increased by acupuncture electric stimulation (AES).[9] PVS in humans is difficult to observe because it collides with its isolation taken from living tissue, but Lee BS, et al (2014) can identify human PVS from human placenta showing positive vWF expression but negative CD31 on PVS endothelial cells indicating not vascular endothelial cells, in addition to negative LYVE-1 expression which proves not lymphatic endothelial cells.[24] PVS can also be identified through the production of antibodies monoclonal using classical traditional methods and then analyzed using cell fusion techniques, hybridoma screening, ELISA, EB, and IF. Four monoclonal antibodies (α-rPVS-m1-1, α-rPVS-m3-2, α-rPVS-m3-4, and α-rPVS-m4-6) were identified by Zhang L. et al. (2020) and characterized using ELISA, EB, and IF methodologies. In α-rPVS-m1-1 and α -rPVS-m4-6 showed a strong affinity for PVS on ELISA and WB examinations, but did not show specificity on IF. Meanwhile, α-rPVS-m3-2 and α-rPVS-m3-4 had strong affinity for ELISA and specificity for IF but almost no response to WB. In α-rPVS-m3-2 and α-rPVS-m3-4 which are stained mainly in the extracellular matrix and cell membranes of rat PVS can be used to differentiate them from blood and lymph vessels.[14]

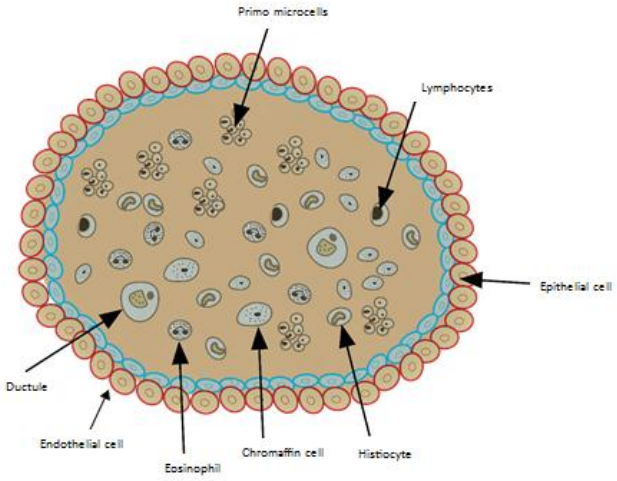


Figure 3. Illustration of various cellular structures inside of the Primo Node in PVS.

Many different immune cell types, including neutrophils and macrophages, are present in PVS. These immune cells migrate to the site of inflammation and accumulate in the primo node. Based on this, the PVS also plays a role in the innate immune system.[11] The presence of chromaffin cells at acupuncture points with PVS fluid suggests that this system might be an endocrine organ that can carry adrenaline and noradrenaline.[29] In addition, the heart, extra-embryonic arteries, and intramembranous vessels are hypothesized to develop later than PVS in the vitelline membrane of the egg. Early embryological studies have shown that the link between the heart and embryonic PVS is one of a matrix for vascular development.[3] In their findings, Scholkman F. et al (2019) found red structures in primo vessels and primo nodes that are similar to erythrocytes, thus suggesting that PVS on the surface of the rat small intestine may have extramedullary hematopoiesis.[7]

CONCLUSION

Primo Vascular System is a circulatory system in the body in addition to the blood circulation system and lymphatic system. In identifying PVS, it is important to pay attention to the isolation of PVS which can be done with or without staining depending on the location of the PVS. After that, further identification can be carried out by histological examination, immunofluorescence, gene expression analysis, Western blot, and others. PVS plays a role in tissue regeneration, as innate immunity in inflammatory processes, as well as extramedullary hematopoiesis.

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