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RESEARCH ARTICLE

Potential Mechanisms of Interior Lymphatic Vessel Primo Vessels in Tissue Repair

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ABSTRACT
The Primo Vascular System was discovered by Bong Han Kim in the 1960s when searching for an anatomical correlate of the acupuncture meridians used in eastern medicine. The Primo Vascular System is a systemic network of thread-like Primo-vessels with intermittent enlargements known as Primo-nodes. Primo-vessels are difficult to view under a microscope due to their small diameters (20-50 µm) and translucent appearance. Primo-vessels have a porous outer membrane that encapsulates small channels named Primo sub-vessels filled with flowing fluid. Primo-vessels are classified into six sub-types based on their anatomical location. The physiological mechanisms of Primo-vessel function are not clear. There are multiple hypotheses based on Primo-vessel and Primo-node structure and cell content, however, supportive functional experimental data is lacking. This review focuses on the "interior" lymphatic vessel Primo-vessel (ILVPV) sub-type, the techniques that are used to visualize them, and experimental studies that attempt to unravel their physiological role after inflammatory stimulation. Speculative hypotheses are presented regarding the handling of signals by ILVPVs for intercellular communication between injured cells and cells stored within "interior" lymphatic vessel Primo-nodes (ILVPNs). One of the stored cell types that are of interest for tissue repair are very small embryonic-like cells. Very small embryonic-like cell activation may be induced by biophoton signals emitted by injured cells and transmitted to ILVPNs via Primo-vessel and/or ILVPV networks. An alternative or additional method for intercellular communication may involve the release of signaling proteins and/or extravesicular bodies carrying genetic messages (i.e., exosomes) by cells in injured tissues. As these signaling factors enter the lymphatic circulation, porous ILVPVs filter them out and transport them to ILVPNs where they initiate very small embryonic-like cell activation to start the tissue regenerative process. Primo Vascular System research will require more physiological functional studies to elucidate the role of ILVPVs and ILVPNs in tissue regeneration. To achieve this goal, future mechanistic studies will need novel biomarkers and animal models.

Key words: Primo Vascular System, interior lymphatic primo vessels, exosomes, stem cells, biophoton

Introduction

In the age of molecular biology and regenerative medicine, a novel systemic anatomical network known as the Primo Vascular System (PVS) has come to light. PVS research has been primarily conducted by South Korean investigators and a small number of international researchers. Bong-Han Kim, a North Korean professor at the Pyongyang Medical University of the Democratic People's Republic of Korea, first discovered the PVS in the early 1960s [1-3]. Kim's institution closed, and his research endured a 40-year hiatus until Kwang-Sup Soh and his colleagues resurrected Kim's work in the early 2,000s. Soh's group has systematically reproduced many of Kim's findings using standard histological methods, and modern imaging and molecular techniques [4]. They have verified the presence of a third circulatory systemic network of thread-like Primo-vessels (PVs) interspersed with Primo nodes (PNs) present in major organs, tissues, blood and lymphatic vessels [5-8]. PVs have been classified into six subtypes (Table 1) depending on their anatomical location and characteristics. However, all PV subtypes have been difficult to study anatomically and physiologically because they have a very small radius and are translucent and invisible to the naked eye. Furthermore, experimental animal models or techniques to study mechanisms of PV function on a temporal basis under normal or pathological conditions need to be developed. To date, the majority of PVS research has focused on the morphological and cellular characterization of PVs. Few manuscripts with molecular data support postulated mechanisms of PV physiological function.

Table 1: Modern Classification of PV Subtypes According to Anatomical Location

PV Subtype	Anatomical Location
Interior	Inside blood and lymphatic vasculature
Interior/Exterior	Along surface of abdominal and thoracic organs
Exterior	Parallel to blood vessels, nerves or found independently
Neural	Ventricles of the brain and subarachnoid space
Intra-Organic	Inside organs
Superficial	Inside the corium and subcutaneous tissues, correlate with Acupoints according to Kim

This review provides a brief overview of the PVS, and primarily focuses on the "interior" lymphatic vessel PV (ILVPV) subtype, which "floats" inside the lumen of lymphatic vessels (LVs) with interspersed "interior" lymphatic vessel PNs (ILVPNs) (Fig. 1). It summarizes the various staining methods utilized to identify ILVPVs, describes findings of the cellular content in ILVPVs and ILVPNs, and recaps experimental results of studies that examined ILVPV physiological function. Also, speculative hypotheses regarding the mechanistic role of ILVPVs and ILVPNs in facilitating remote intercellular communication between injured cells and stem cells to initiate tissue repair are presented. These include the potential role of the ILVPV network in tissue repair by transmitting cell-generated ultra-violet (UV) biophoton signals from injured tissues to undifferentiated cells stored in ILVPNs, and/or their ability to gather and transport signaling proteins, and extra-vesicular vessels to facilitate intercellular communication between injured cells and cells stored in ILVPNs to initiate local immune responses or tissue regenerative processes.

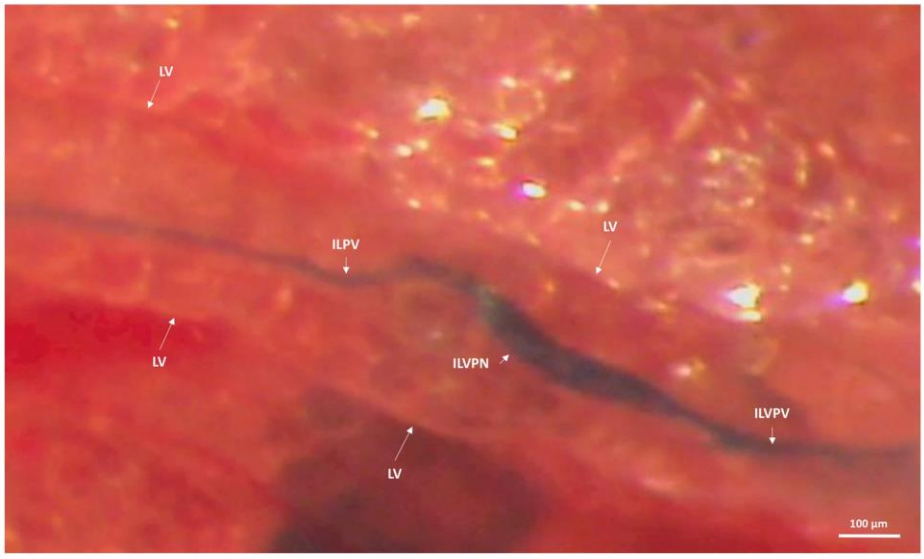

Figure 1. Intravital microscopy image of an ILVPV and ILVPN stained with Alcian blue inside a lymphatic vessel (LV) in the rat abdominal cavity. Notice the larger diameter of the ILVPN.

The Primo Vascular System

Bong-Han Kim discovered the PVS while searching for the anatomical correlate of the acupuncture meridians. Kim originally named PVs "*Bong-Han Ducts*" and PNs *Bong-Han Corpuscles* [1]. However, in 2010 at the first International Symposium on the new era of PVS research, the Bong-Han System nomenclature was renamed to the current terminology [4]. Prior to Kim's mysterious disappearance, he published five reports in which the structure and presumed function of PVs and PNs were described in detail. Kim believed that the PVs were conduits of flowing Primo fluid (a new circulatory system) and electrical impulses, and also indicated that PNs contained "sanals" (regenerative cells) that were involved in tissue repair. Kim described PVs as bundles of 10-20 Primo sub-vessels (PSub-Vs) or small channels wrapped by a porous endothelial membrane. The size of PVs ranged between 20 to 50 µm in diameter. PN size ranged between 0.05 to 1.0 mm in diameter and is composed of a high-density web of intersecting PSub-Vs that appear to connect to the relatively large "sinus" compartment where various types of lymphocytes and regenerative cells reside [1,3].

Modern-era PVS investigators have verified most of Kim's anatomical descriptions of the various PV subtypes [9,10]. Kim was forward-thinking since the regenerative stem cell biology principles were in their infant stage. Kim aptly described the role of PNs as regenerative organs containing stem cells for tissue repair [1]. Indeed, recent studies have reported that PNs contain undifferentiated cells that express stem cell markers or are like very small embryonic-like cells (VSELs) [11-15]. PVs have Primo fluid flowing through the PSub-Vs and PN structures, and the fluid flow within PSub-Vs can range between 100 and 800 µm/s [10,16]. Fluid flow within PSub-Vs suggests that signaling proteins and extra vesicular bodies (i.e., exosomes or apoptotic bodies) involved in intercellular communication are transported by the PVS to potentially deliver messages to cells stored in PNs [17].

Kim's original reports listed six PV subtypes, however, modern PVS investigators have only identified five of the six [18]. In mammals, PVs have been found under the skin, inside and on the outer surface of organs, in the brain, spinal column, and inside the blood and lymphatic vessels [1,3,19-21]. Studies in immunocompromised mice with human cancer xenografts have reported increases in the density and prominence of PVs in tumors and surrounding tissues, leading investigators to speculate that the PVS plays a role in the transport of cancerous cells and the development of metastasis [22-24]. Although these are interesting findings, the role of the PVS in cancer metastasis is beyond the scope of this review.

Characterization of ILVPVs

The re-emergence of PVS research has focused primarily on visualizing and characterizing the various PV subtypes reported by Kim. The majority of studies describe the utilization of different dyes and techniques to provide contrast to the five PV subtypes, including ILVPVs [7,9,10,25-29]. Once an ILVPV has been stained with a dye and visualized with the aid of a microscope, the PV is harvested and immunohistochemical techniques are utilized to further identify the structure. There is a

greater preference for the utilization of various blue dyes to identify ILVPVs, perhaps related to the use of a blue dye by Kim, which he never described. Blue dyes that have been used include Alcian blue, Trypan blue, and Toluidine blue [5,7,15,25-27,30,31]. Alcian blue is the most popular because it provides a nice dark blue contrast to ILVPVs and it has a high affinity for hyaluronic acid which is present in PSub-V Primo fluid. However, Alcian blue has some drawbacks, it requires meticulous handling to prevent aggregation of its particles, and when injected *in vivo* into lymph nodes, it takes hours to clear from LVs to allow the visualization of ILVPVs. Nevertheless, when all blue dyes were compared head-to-head, Alcian blue proved to be superior to Trypan blue and Toluidine blue [27]. Trypan blue easily washed out from the LV wall and ILVPV membranes, and Toluidine blue left patchy inconsistent stains and leaked out from LVs into surrounding tissues [27].

Various contrast-providing agents other than blue dyes have been tried to successfully visualize ILVPVs *in vivo*. A technique that uses Janus Green B (JGB) dye injected into rabbit lumbar lymph nodes reported an ILVPV visualization rate of >85% of the animals studied [9], however, despite its high success rate its popularity among investigators has been low. Other agents include 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) a highly lipophilic red fluorescent dye that strongly stains ILVPVs and traces its path inside lymphatic nodes [29]. Other non-dye approaches include the utilization of nanoparticles. Fluorescent cobalt-ferrite magnetic nanoparticles ~50 nm in diameter were successfully used to infiltrate ILVPVs to provide contrast under fluorescent microscopy [8]. Also, hollow gold nanospheres ranging in size from 50 to 120 nm provided a turquoise/blueish contrast for ILVPV visualization [19]. The preferred injection sites for dye or nanoparticle administration are the inguinal [26-28,31] and lumbar lymph nodes [7-9,19,25,32]. Occasionally, Alcian blue was directly injected into LVs near the caudal vena cava [9,33]. The presence of ILVPVs has been studied in different mammal species. Early studies were performed in rabbits [10,33] and later studies in rats [19,26,27,34] and mice [7,35]. Larger animal species have not been used as animal models for ILVPV research, however, there is a single report of an "interior" PV subtype found inside a human umbilical cord blood vessel [6].

Histological characterization of ILVPVs

Historically, after an ILVPV was visualized with a dye, it was harvested and further analyzed using immunohistochemical techniques to verify its structural morphology. The first analyzed structures were the shape and pattern arrangement of ILVPV cell nuclei after staining with 4',6-diamidino-2-phenylindole (DAPI). A distinctive rod shape nuclei pattern emerges that is different from nuclei patterns observed for lymphatic or blood vessels [35]. Next, phalloidin dye was applied to identify filamentous actin which is present in the endothelial portion of the ILVPV [25]. Following DAPI and phalloidin staining, immunohistochemical techniques can be used to further identify components in the ILVPV. The outer membrane of the ILVPV has outer epithelial and inner endothelial layer characteristics that include the typical markers of epithelial membrane protein-3 (EMP-3), a membrane protein found in epithelial cells, and Von Wille-brand factor (vWF) a membrane protein present on endothelial cells [32]. Dual staining studies for these markers when found on the outer layer of a vessel provide additional support that the structure under study is an ILVPV [32]. To exclude that ILVPVs are not blood or lymphatic vessels immunohistochemical staining techniques utilizing the endothelial cell marker CD31 and the lymphatic endothelial cell marker lymphatic vessel endothelial receptor 1 (LYVE1) have been utilized [6,10,25,35]. However, the use of LYVE1as an exclusionary marker is controversial since more recent studies have reported its expression in ILVPVs [36]. Identifying ILVPVs utilizing distinguishing morphological characteristics or exclusionary immunohistochemical markers is not ideal, and efforts to identify a specific biomarker are needed to advance the field.

The composition of the ILVPV interstitial space has been examined for potential markers. Initially, Kim described the ILVPV interstitial space as a matrix of longitudinal and circular fibers, but he did not identify the fiber type [1]. He hinted that the fibers had an affinity for silver [1], suggesting a collagen composition since silver-based stains were frequently utilized to detect collagen. However, the interstitial collagen content of ILVPVs is controversial. When ILVPVs were analyzed using a combination of x-ray microtomography with Masson Trichrome staining no collagen was found in the interstitial space [33]. However, when analyzed with transmission electron microscopy (TEM) collagen fibers were identified [26]. The discrepancy may be explained by the different techniques used, TEM is more sensitive and provides significantly higher magnification and resolution than x-ray microtomography.

Immunohistological characterization of ILVPNs

The composition of the ILVPN has been inspected for potential clues to its function. Kim described the ILVPN as a spherical or oval-shaped structure with a thin membrane measuring 200-300 μm in size. The PSub-Vs that enter ILVPNs disperse and intertwine inside the reticular tissue forming its internal matrix. ILVPNs have compartments that Kim referred to them as "sinuses" that contain basophile granules, chromaffin granules, and myelopoietic and lymphogenic cells [1]. Over the past three years, efforts using modern molecular techniques to identify a biomarker for the advancement of PVS research have emerged. Shin et al. using RNA-sequencing performed differential expression of genes (DEG) analysis comparing ILVPVs with LVs [37]. Ten potential gene candidates were identified with the goal of developing monoclonal antibodies (mAbs) to construct maps of the PVS connectome (network) in a similar manner as connectome maps of neural connections are generated in the brain [37]. In 2020, Zhang and Oh reported for the first time the development of mAbs specific to PVs and PNs [38]. Exterior subtype PVs and PNs were harvested, their protein content isolated and injected into mice as an immunogen, and then, using classical fusion methods for hybridoma cells, mAbs against PVs and PNs were generated. The two mAbs (named: α-rPVS-m3-2 and α-rPVS-m3-4) were tested in rats and they demonstrated specificity to the extracellular matrix and cell membranes of exterior PVs and PNs, and ILVPVs [38].

Recent analyses of the cellular content of rat ILVPNs found an abundance of cells consisting of mast cells (20%), eosinophils (16%), neutrophils (5%), histiocytes (53%), lymphocytes (1%), and immature stem-like cells (3%) [32]. Some of these cells were positive for norepinephrine and chromogranin confirming Kim's early findings [32]. Additionally, cells expressing hematopoietic stem cell markers CD 34 and cytoplasmic positive octamer-binding transcription factor 4 (Oct4) were found scattered throughout ILVPNs [32]. Analyses of mouse ILVPN content utilizing colony-forming unit-granulocyte-macrophage (CFU-GM) and colony-forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM) assays found hematopoietic progenitor cells (HPCs) capable of producing macrophage and neutrophil precursors; and basophils, megakaryocytes, eosinophils, and erythroblasts, respectively [13]. Also, pluripotent stem cells (PSCs) capable of producing hemangioblast-like cells and various types of HPCs were found in ILVPVs. Many colonies grown under the CFU-GM conditions yielded mostly mast cell progenitors [13]. Notably, cell granules were found within PSub-Vs, suggesting that mast cell degranulation within the ILVPNs is possible, raising further questions [32]. What is the source of the mast cell degranulation signal? What unknown physiological processes may be occurring within ILVPNs? What is clear is that ILVPNs serve as storage compartments for PSCs, HPCs, and hematopoietic cells, and PSub-V fluid flow may transport signaling proteins and extra vesicular bodies (i.e., exosomes, apoptotic bodies) with genetic information to ILVPNs where they can interact with stored cells to facilitate immune responses and regenerative processes.

ILVPV and ILVPN Physiological Function

Few studies have performed mechanistic studies in ILVPVs to examine their potential physiological role. Electrical signals have been recorded from interior/exterior PV subtypes [39], however, the conduction of electrical signals by the ILVPV subtype has not been reported. Characterization studies of cells within ILVPVs and ILVPNs have described the presence of myeloid hematopoietic progenitors for potential immune function as well as VSELs for tissue regeneration [11,13,15,32]. The restorative effect of VSELs harvested from ILVPNs and "interior" intra-venous PNs (IIVPNs) was demonstrated in a mouse model of cerebral hypoxic ischemia combined with a brief period of systemic hypoxia [11]. VSELs were isolated, induced to a neuronal stem cell phenotype, expanded in vitro, and injected intravenously to mitigate the effects of ischemia/reperfusion injury. Brain infarct size was significantly reduced in VSEL-treated mice [11]. Interestingly, the density of VSEL-like cells in ILVPNs was significantly greater compared to an equal mass of bone marrow, suggesting that VSEL ILVPN content "pound-for-pound" has potentially a larger regenerative capacity than bone marrow [11].

One of the more elegant mechanistic/ functional studies in the PVS literature was performed by Choi and colleagues [40]. Their group performed cellular profiling and secretome analysis of external subtype PVs and PNs. PV samples were harvested from the peritoneal cavity lateral wall, intestine, or liver and analyzed under normal conditions and locally mediated inflammation induced by lipopolysaccharide (LPS) or zymosan. They found that PNs primarily harbored myeloid cells (TER119^{+} red blood cells (RBCs), Gr-1^{High} neutrophils, and F4/80^{Low} macrophages) in both normal and LPS-activated states [40]. With proinflammatory stimuli, various types of inflammatory cytokines and chemokines were secreted by these cells, and simultaneously, the volume of PNs expanded with increasing numbers

of neutrophils and macrophages [40]. The results suggest that local inflammation induces migration and accumulation of macrophages and neutrophils into PNs, where inflammatory cytokines and chemokines are amplified, enhancing signaling and alerting the systemic immune system to the presence of an inflammatory site [40]. Apparently, exterior PVs and PNs in mice serve as a component of the lymphoid system for innate immunity signaling with an intermediary role in cell-mediated local inflammation. However, their immune role in aging mice (>7 weeks old) is not clear since exterior subtype PVs and PNs degenerate into thinner thread-like structures with fewer PNs [40]. Choi's group compared the same age wild type C57BL/6 mice with immunodeficient C57BL/6 mice lacking in recombination-activating genes 1 ($RAG1^{-/-}$) to test the hypothesis that immunodeficient mice as they aged would retain their PV and PN morphology since the knockout mice have an undeveloped lymphoid system with no mature B and T lymphocytes [40]. Temporal repeated comparative measurements of PN diameters demonstrated that PNs in 8-week-old $RAG1^{-/-}$ mice were significantly larger than in control mice, and PNs of immune-deficient mice at 10 weeks of age remained prominent and easily visualized [40]. Thus, the preservation of PVs and PNs in aging $RAG1^{-/-}$ mice suggests that local immune responses mediated by PVs and PNs are retained to provide some protection that would be essential to their survival. Alternatively, degeneration of PVs and PNs with age would reduce local immune signaling perhaps delaying the systemic immune response, making older mice more susceptible to infection or cancer.

Administration of LPS into rabbit LVs or lymph nodes to examine the role of ILVPVs and ILVPNs in response to inflammation is becoming a popular model [15,36,41-43]. Lee et al. using rabbits, combined LPS stimulation to enhance the size of ILVPVs for easier visualization with the fluorescent dye diaminobenzidine (DAB), which under fluorescent microscopy, using green and red filters, allows the tracking of the vessels for the construction of a PVS connectome map [15]. The authors postulate that LPS treatment should also stimulate the acupuncture meridians, and this staining technique will allow the study of structural and functional changes of ILVPVs under inflammatory conditions, which may provide information as to their physiological function [15]. The expression patterns of lymphatic endothelial cell (LEC)-related genes after LPS stimulation and acupuncture electric stimulation (AES) at two major acupoints Joksamni (ST36) and Hapgok (LI04) were studied in rabbit LVs and ILVPVs [36]. Under inflammatory conditions, the genes fms-related tyrosine kinase 4 (FLT4), LYVE-1, prosper homeobox protein 1 (PROX-1), and podoplanin (PDPN) were highly expressed in ILVPVs compared to that of LV endothelium, suggesting that ILVPVs have greater involvement in the inflammatory response [36]. Under ST36 and LI04 acupoint stimulation, lymphatic-related genes including metal-response element-binding transcription factor 2 (MTF2), hypoxia-inducible factor alpha (HIF1α), angiotensin II type 1 receptor (AGTR1), and angiotensin II type 2 receptor (AGTR2) demonstrated an overall increase in ILVPVs also inferring a greater involvement than LVs after AES [36]. It appears that the expression of LEC-related genes in ILVPVs is enhanced under inflammation and AES compared to LVs, and the authors suggest that one of these genes could serve as a biomarker to evaluate the effects of AES under pathophysiological conditions [36]. Comparative rabbit studies on isolated rabbit ILVPVs and LVs utilizing RNA sequencing and DEG analyses following LPS stimulation demonstrated that the genes FLT4, heat shock protein 1 (HSPH1), and erythropoietin-producing hepatocellular carcinoma B2 receptor (EPHB2) were highly expressed in ILVPVs [41]. This diverse gene expression suggests that ILVPVs may be involved in the regulation of various pathophysiological processes [41]. In a recent study, Shin's group examined gene expression of lymphocyte markers in isolated rabbit ILVPVs and the combination of LVs+ILVPVs. The master control gene for lymphangiogenesis and fate of LECs, prospero homeobox 1 (PROX-1), along with the master transcriptional regulator of cellular and developmental responses to hypoxia, hypoxia-inducible factor 1 alpha (HIF1A) were investigated [42]. RNA-sequencing and quantitative polymerase chain reaction (qRT-PCR) analyses were performed, and a library was constructed for DEG analysis. Following LPS treatment, mRNA transcriptional data demonstrated an increase in PROX-1 and HIF1A expression in ILVPVs, however, in comparative DEG analysis a decrease in expression was observed in LVs+ILVPVs. Under LPS stimulation and AES (at acupoints ST36 and LI04), gene expression was reduced, suggesting gene inactivation by acupuncture [42]. The authors suggest that PROX-1 and HIF1A may play a role in the LEC response and enlargement of ILVPVs observed following LPS stimulation. Monitoring the expression of these genes can be useful in understanding the functional role of ILVPVs in pathophysiological studies. The latest rabbit study that used LPS injections into peritoneal lymph nodes reported that when administered in combination with the COVID-19 immunotherapeutic antibody Foralumab ILVPVs

were further enlarged than when LPS was used alone [43]. The goal of the study was not clear; however, the authors indicate that in the presence of inflammation the antibody-induced immune response elicited greater structural changes, and thus, ILVPVs may play a central role in modulating immune responses.

ILVPVs as waveguides for biophoton signaling for intercellular communication

Given that many fundamental questions about the function of the PVS are still open, it seems pertinent to explore the physiological utility of the PVS network in conveying signals between groups of cells employing a modality of communication other than conventional modes (i.e., electrical signaling, exosomes, or signaling proteins). It has been suggested that the PVS may serve as a type of fiber-optic signaling network that aids in the transmission of encoded biophoton signals trapped and emitted by deoxyribonucleic acid (DNA) for intercellular communication [18,44]. This concept is not farfetched since cells are known to emit quanta of light in the form of biophotons spanning the near-UV to near-infrared frequency range [45-48]. The initiation of biophotons in different tissues by multiple organisms including humans is well-recognized [49,50]. The idea that UV biophotons carry encoded signals to exchange information between biological systems is an old concept that is regaining recognition [51,52]. Soh has proposed that the translucent properties of PVs and their collagen outer cover envelope may serve as "photonic waveguides" for the transmission of biophoton-encoded signals to remote cells [44,46]. The feasibility of a faster modality of intercellular communication between neurons has been proposed in the brain where myelinated axons not only conduct electrochemical signals but also serve as waveguides for neuron-emitted biophotons [45]. Soh's postulate may help explain the rapid systemic effects observed when acupuncture needles are applied to patients. Indeed, biophoton emission by cells has been observed experimentally after stress induction by mechanical disruption of cells, exposure to ionizing radiation, or oxidative metabolic processes [45,46,48]. Information transfer via biophoton signaling would be significantly faster than electrical neural signals, and would not be affected by heat noise or other common disturbances [45].

A growing body of literature provides evidence that UV biophotons play a role in intercellular communication [47,48,53,54]. The concept was first demonstrated in experiments between different cell types in which human keratinocytes exposed to UV radiation released microRNA-carrying exosomes that targeted melanocytes and modulated their pigmentation by altering melanin gene expression and enzymatic activity [53]. UV biophoton-mediated intercellular communication between similar cell types has been demonstrated in what is known as the "bystander effect." In this context, cells exposed to UV irradiation synthesized and released exosomes that fused with naïve "bystander cells" (non-irradiated cells), and these cells in turn released exosomes with similar RNA and protein cargo as the UV irradiated cells, which fused with other naïve cells, further expanding the population of "bystander effect"-expressing cells [54,55]. A higher level of complexity of the bystander effect was demonstrated by Le et al. using ionizing radiation to induce the emission of UV biophotons by human keratinocytes, which in turn elicited an effect on naïve keratinocytes cultured separately at a distance [47]. Cultured HaCaT human keratinocytes incubated with tritium, emitted UV biophotons that induced the release of exosomes by naïve bystander cells cultured 1.5 cm apart without cell-to-cell contact or the sharing of media [47]. The experiment was repeated using colon carcinoma cells and the bystander effect was observed again, suggesting that remote UV biophoton transmission is a modality of intercellular communication [56]. Therefore, it is plausible that the PVS systemic network can transmit biophoton-coded messages originating from various cell sources in distress or to regain homeostasis. Presumably, these signals would traverse through ILVPVs to ILVPNs, and transmit signals that affect cells residing within ILVPNs whether by inducing the release of exosomes to activate stem cells and/or by directly inducing the differentiation of stem or immune cells to initiate immune responses or tissue regeneration processes.

Potential mechanisms by which ILVPVs and ILVPNs initiate tissue repair

The basis for the involvement of ILVPVs and ILVPNs in tissue regeneration/repair is Kim's description that PNs contained "Sanals" (stem cells) [1], and the modern era findings establishing that undifferentiated cells and/or VSELs are present within PNs [11-14]. The mechanisms by which ILVPVs and ILVPNs collect and congregate cell signals released by stressed cells to initiate stem cell activation and migration from ILVPNs to the site of injury are not known. As stated earlier, it is plausible that intercellular communication mediated by local ILVPVs transmitting biophoton signals, originating from injured cells, is received directly by stem cells in ILVPNs to initiate their activation and release.

Another plausible mechanism involves the application of the old biology adage "structure determines function". It is well established that ILVPVs "float" freely in the flowing lymphatic fluid of LVs, and there must be a reason for this unusual anatomical feature. Injecting hollow gold nanoparticles of similar size as exosomes and extravesicular bodies (50 to 120 nm) into the lumbar lymph nodes penetrated ILVPVs and ILVPNs staining both structures [19]. Thus, it is plausible that exosomes circulating in lymphatic fluid should be able to penetrate ILVPVs via their pores, be transported to ILVPNs via PSub-V fluid flow, and carry genetic messages to cells residing within. ILVPNs harbor VSELs, HPCs, PSCs, mast cells, macrophages, and neutrophils [11,13,40], and local tissues can release signals that are collected by ILVPVs and transported to ILVPNs to interact with stored cells. Immune cells including macrophages and neutrophils which secrete cytokines and chemokines can amplify immune response signaling in a similar manner as it occurs in the lymph nodes. Thus, a reasonable mechanistic hypothesis as to how local ILVPVs and ILVPNs may mediate tissue repair is presented in Fig. 2. As tissues are stressed or injured, local or infiltrating cells release into the interstitial fluid chemokines, cytokines, and extra vesicular vesicles (i.e., exosomes and or apoptotic bodies carrying genetic messages) that eventually are transported to local LVs by fluid flow. Porous ILVPVs can filter out all these signaling factors from the lymphatic fluid and transport them to ILVPNs via PSub-V fluid flow. The signaling proteins and exosomes accumulate within ILVPNs and interact with stored VSELs to initiate their activation. Once VSELs are activated, they detach, migrate out of ILVPNs via out-flow PSub-Vs, and eventually exit via ILVPV pores into the lymphatic circulation. The lymphatic flow carries the activated VSELs to the heart's right atrium where they mix with circulating blood. At the site of tissue injury, the endothelium of venules is activated due to the presence of proinflammatory factors, and chemokines released by local cells attract and facilitate the extravasation of activated VSELs to initiate tissue repair. Future mechanistic studies regarding the role of ILVPVs and ILVPNs in tissue regeneration are needed. They will require the development of a novel tissue injury animal model that would permit repeated temporal measurements of changes in ILVPN cell content, and the use of adaptive transfer of labeled-VSELs (from green-fluorescent transgenic mice or rats) into wild type animals to track their homing and ability to repair injured tissues.

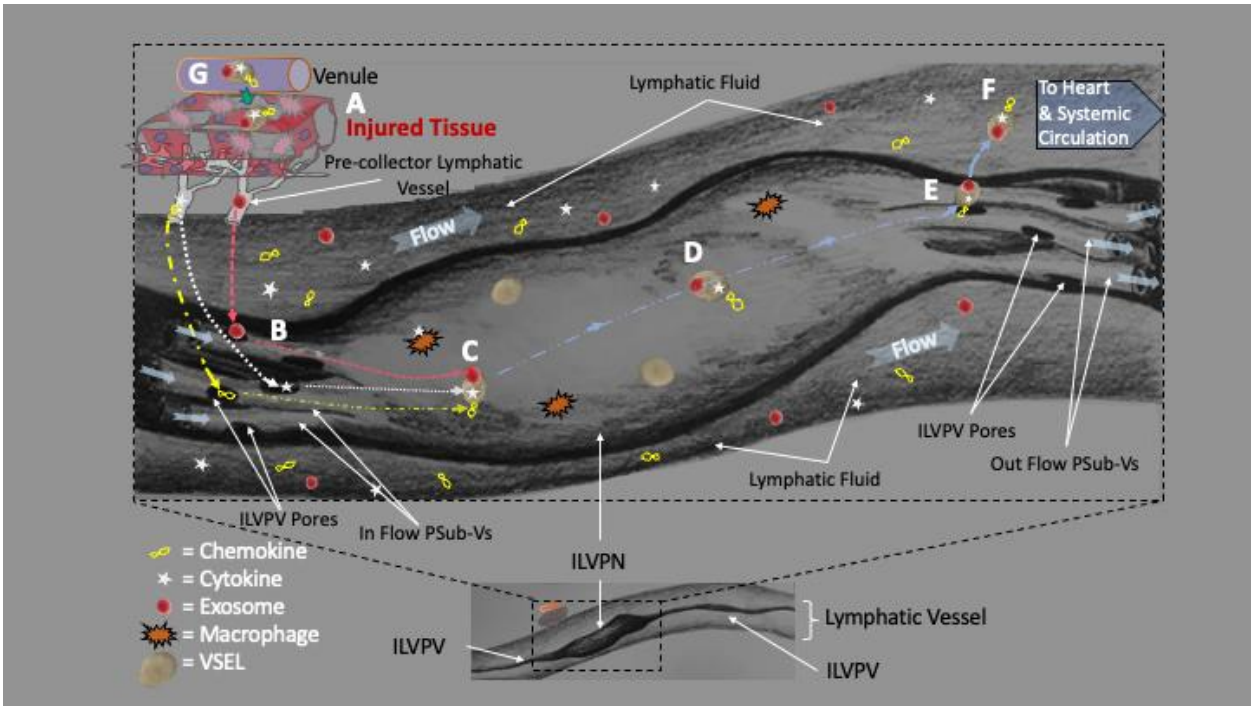

Figure 2. This cartoon illustrates the potential role of ILVPVs and ILVPNs in facilitating intercellular communication between cells from injured tissues and VSELs stored in ILVPNs. The bottom panel illustrates a low-magnification image of an LV with a stained ILVPV, and the top panel is a magnified rectangular inset. (**A**) Local cells and immune cells from injured tissue release exosomes, chemokines, and cytokines into the interstitial space fluid, which is transported to LVs by pre-collector lymphatic vessels. (**B**) As exosomes and

signaling proteins are carried by the lymphatic flow, they enter ILVPVs through their porous membranes and are transported to ILVPNs via PSub-V fluid flow. (**C**) At ILVPNs, the signaling proteins and exosomes converge to interact with stored VSELs initiating their activation. (**D**) Activated VSELs detach from ILVPN sinuses and are carried by *PSub-V fluid* flow towards outflow PSub-Vs. (**E**) Activated VSELs exit the outflow PSub-Vs into the circulating lymphatic fluid via the ILVPV pores. (**F**) The lymphatic flow carries activated VSELs to the heart, where they mix with blood, and circulate systemically. (**G**) At the site of tissue injury, pro-inflammatory factors will activate the endothelium of venules to allow VSEL extravasation to begin tissue repair.

Conclusion

It is evident from modern research techniques that the PVS is a complex systemic network that appears to have various important physiological functions including tissue regeneration. Novel techniques and tools to overcome the challenges presented by the small size and translucent nature of ILVPVs need to be developed. Also, identifying biomarkers specific to PVs and PNs will facilitate mechanistic studies that will be essential to advance the field. The potential for the PVS network to serve as waveguides for the transmission of biophoton signals for remote intercellular communication to activate stem cells may explain how acupuncture works systemically to promote tissue repair. However, novel techniques will need to be developed to study biophoton signaling mechanisms via the PVS network. The mechanistic hypothesis presented as to how local ILVPVs and ILVPNs function as regenerative organelles offers a logical explanation for their presence inside LVs. Innovative mechanistic studies focusing on the pathophysiological role of ILVPVs and ILVPNs in facilitating intercellular signaling for tissue repair via biophotons, extra-vesicular bodies, and signaling proteins warrant further investigation.

Conflict of Interest

The authors have no conflicts of interest to declare.

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