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TECHNICAL NOTES

Protocol for Detecting the Primo Vascular System in the Lymph Ducts of Mice

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Abstract
The primo vascular system (PVS), which is the proposed conduit for the acupuncture *Qi*, is a complex network distributed throughout an animal's body. However, even with a microscope, it is not easily detectable because of its transparency. Thus, its existence is largely unknown in current anatomy. A convincing demonstration of its existence is needed. The lymph-primo vessel (PV), which is a subsystem of the PVS, is a very effective visual demonstration of the PVS. The lymph-PVS is a mobile threadlike structure floating in lymph ducts that has been observed in rabbits, rats, and mice by several independent teams. The involved techniques are novel and rather complicated; therefore, we have already provided detailed protocols for the surgery; for the injection of the staining dye; and for the detection, extraction, and identification of the PVS in rabbits and rats. However, the mouse is one of the most important laboratory animals used for various biomedical research purposes. For the convenience of researchers who wish to initiate the PVS experiments in mice, we provide a shortened version of the protocol, despite many similarities with previously published protocols. Thus, researcher can easily obtain the samples of the lymph-PVS of mice.

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1. Introduction

The primo vascular system (PVS) is being established as a new circulatory system that is distributed throughout an animal's body, including humans [1]. It was first discovered in the 1960s by Bong-Han Kim as an anatomical structure that corresponded to acupuncture meridians and is the conduit of the so called *Qi* of Traditional Chinese Medicine [2]. It was not confirmed until the year 2002 when serious investigations on the PVS began. The main reason for the difficulty of detecting the PVS in an animal's body is its transparency and small size. The PVS is composed of primo vessels (which are approximately 20—50 μm thick) and primo nodes (which are oval and approximately several hundred micrometers). The fluid flowing in the PVS is called the primo fluid. The primo vessels, nodes, and fluids are all transparent and therefore very difficult to detect without special techniques and experimental skills [3].

The PVS has been detected in various internal organs of mice, rats, rabbits, dogs, pigs, and humans [4]. Most visual and direct confirmations of the PVS has been by the aid of staining agents such as fluorescent nanoparticles [5] or Alcian blue [6] to observe the mobile threadlike PVS floating in the lymph. The PVS is observable *in vivo* and *in situ* in lymph vessels such as the thoracic ducts and vessels between the inguinal and axillary lymph nodes.

According to Kim [2], important functions of the PVS are hematopoiesis and regeneration of damaged tissues. This idea is supported by the recent detection of hematopoietic stem cells [7] and, more importantly, small embryonic-like stem cells in the PVS [8,9].

For researchers who want to reproduce the experiments, we have already presented a series of protocols for observing the PVS in the lymph vessels of rabbits [10] and rats [11,12]. Because of the importance of stem cells in the PVS, we present the current protocol for observing the PVS in the lymph vessels of mice. We used mice because stem cell research is mostly performed with mice rather than rats or rabbits. This protocol would be convenient for potential start-up researchers, even though it largely overlaps with previously published protocols.

2. Materials

2.1. Equipment and setup

2.1.1. Microscopes and light source

The following microscopes and light source are used: a stereomicroscope (SZX12; Olympus, Tokyo, Japan) with a charge-coupled device (CCD) camera (DP70; Olympus, Tokyo, Japan); a phase contrast microscope (BX51; Olympus, Tokyo, Japan) with a CCD camera (Infinity 3; Lumenera Corporation, Nepean, Canada); and a halogen lamp (KLS-100H-LS-150P; Kwangwoo Co, Ltd, Pohang, South Korea) for the light source and optical fiber illuminator (KLS-100H-LS-150P; Kwangwoo Co, Ltd, Pohang, South Korea).

2.1.2. Surgical instruments

Surgical instruments and ophthalmic surgical instruments by Tumed (Rotwildstraße, Germany) are used. The following equipment are also used: electric surgical unit (Surgitor, Korea); electrocautery (Umeco, Seoul, South Korea); Pet Specialty cordless trimmer (Oster Professional, Burns, USA); disposable Gentax latex gloves (Geneall Biotechnology, Seoul, Korea); masking tape (Scitech Korea Inc., Seoul, Korea); gauze (Scitech Korea Inc., Seoul, Korea); surgical drapes (Scitech Korea Inc., Seoul, Korea); and an electrical heating pad (30 mm × 30 cm; Woojin Tech, Seoul, Korea).

2.1.3. Syringes and filters

Syringes and filters used are the following: hypodermic syringe (Kovax-Syringe; Korea Vaccine Co., Seoul, Korea); BD ultrafine insulin syringe, 31G (Becton, Dickinson and Company, Franklin Lakes, NJ, USA); BD filter syringe, 5 mL (Becton Dickinson Medicals Ltd., Singapore); BD filter syringe, 10 mL (Becton Dickinson Medicals Ltd.); glass microfiber filters, 110 mm (GE Healthcare Co., Buckinghamshire, UK, cat. no. 1820-110); and hydrophilic minisart syringe filter (Sartorius Stedim Biotech, Göttingen, Germany).

2.1.4. Staining and histology instruments

The staining and histology instruments used are the following: pH meter (Thermo Electron Corporation, Waltham, MA USA); glass funnel (Dongsung Science, Seoul, Korea); round bottom test tube (5 mL; BD Falcon, San Jose, CA, USA); Coplin jar (Fisher Scientific, Hampton, NH, USA); PAP pen (Invitrogen, Waltham, MA, USA); Vortex-2 Genie mixer (Scientific Industries, Bohemia, NY, USA); from 5-μL to 10-μL Finnpipette (Sartorius Korea Biotech Co. Ltd., Seong-Nam, Korea); disposable transfer pipette (Lappia, Korea); microslides (silane coated; 76 mm × 26 mm; Mutopure Chemicals Co, Ltd, Tokyo, Japan); 100 deckglaser cover slips (24 mm × 50 mm; Knittel Glass, Brunswick, Germany); and Leica CM1800 cryostat (Leica, Nussloch, Germany).

2.1.5. Dissecting instruments

The following dissecting instruments are used: two large scissors; a small microscissor; two large forceps; two microdissecting tweezers; small forceps; one pair of fine straight forceps; one pair of curved forceps; one microdissecting straight forceps; one pair of angular microdissecting forceps; and one 31 G insulin syringe (Fig. 1).

Caution!

1. All instruments and other equipment must be sterilized before use.
2. To reduce the chances of contamination when proceeding through the tissue layers, use separate sets for the skin and the peritoneal wall, and for dissecting and extracting the primo system in the lymph vessels.

2.2. Reagents and setup

2.2.1. Experimental animals

Seven-week-old ICR male mice (30—32 g) were purchased from DooYeol Laboratory Animal Company (Seoul, Korea). Males are preferred because they develop less abdominal fat, which makes the surgery easier. The animals are housed in a constant temperature-controlled environment (23°C) with 60% relative humidity. All animals are exposed to a 12-hour/12-hour light-dark cycle and have *ad libitum* access to food and water. Procedures involving animals and