Flap management strategies: real-time laser speckle based blood flow monitoring and polydeoxyribonucleotide injection

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Directed by Professor Yoon Woo Koh

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ABSTRACT

**Flap management strategies:
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This study proposes a dual strategy for the early diagnosis of vascular abnormalities using real-time laser speckle imaging and protection of flaps from ischemic necrosis using the administration of PDRN which promotes neovascularization in the flaps. In the murine bipedicled skin flap model, vessel ligation groups and normal groups were established. Flap necrosis severity was assessed through visual inspection and compared with laser speckle imaging findings. When setting the cutoff value for the blood flow index at 12.5 in ROC curves, laser speckle imaging demonstrated a diagnostic performance for flap necrosis prediction with a sensitivity of 96% and specificity of 98.8%. In the murine random skin flap model, the extent of flap necrosis was compared between PDRN injection groups and PBS injection group. The surviving flap area ratio was significantly higher in the PDRN group than in the PBS group. Additionally, the PDRN group exhibited statistically lower expression of IL-1β and significantly higher expression of VEGFα compared to the PBS group. The results of this study indicate that subcutaneous injection of PDRN in animal skin flap models is effective in enhancing flap survival during flap necrosis development. Furthermore, the study provides fundamental data suggesting that the laser speckle imaging system can be a valuable imaging tool for early diagnosis of flap perfusion abnormalities.

Key words : polydeoxyribonucleotides; surgical flaps; laser speckle contrast imaging

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I. INTRODUCTION

Random skin flaps are frequently employed in reconstructive surgery, establishing connections with the subdermal plexus via a delicate, unnamed peripheral artery responsible for blood supply.[1)] These flaps offer advantages in terms of color and texture matching to the surrounding tissue, reducing donor site complications and allowing reconstruction under a single surgical field of view.[2)] However, their application for large defects can be challenging, and the risk of necrosis in the distal part of the flap due to insufficient blood supply remains a major concern.[3)] Ischemic necrosis is the most frequent and severe complication associated with flap surgery, occurring when adequate angiogenesis does not develop laterally from the base of the flap.[4)] The clinical efficacy of various pharmacological interventions designed to forestall ischemic necrosis remains a topic of controversy.[5,6)] Thus, the vigilance of postoperative flap monitoring remains indispensable, facilitating the early detection of thrombosis and facilitating timely salvage surgery during the optimal golden period.

Advancements in biomedical imaging technology have led to non-invasive monitoring methods such as ultrasound doppler surface monitoring, color doppler sonography, and laser doppler flowmetry. However, the flap monitoring still relies on clinical experiences of medical staffs (residents or nurses) because there may be no single reliable technique.[7)] A potential solution is the utilization of laser speckle contrast imaging (LSCI), which

enables the evaluation of microvascular flow by analyzing the interference pattern created by scattered light when a laser illuminates the tissue.[8] LSCI is non-invasive, safe for use on most parts of the body, and allows real-time monitoring of microcirculation, providing consistent blood flow quantification regardless of the user's expertise with the device.[9]

Polydeoxyribonucleotide (PDRN) represents a DNA fragment derived from salmon sperm DNA, possessing an impressive 95% similarity to human DNA and boasting tissue regeneration and anti-inflammatory properties.[10] The present study seeks to explore the protective potential of PDRN against ischemic necrosis and real time LSCI monitoring for evaluating blood flow dynamics in a murine model for managing skin flaps and preventing ischemic necrosis.

II. MATERIALS AND METHODS

1. Establishment of murine bipedicled skin flap model

This animal study was conducted with an approval from the Institutional Animal Care and Use Committee at the Yonsei University Wonju College of Medicine (Protocol YWC-170313-1). The experiment used 10 male Spraque-Dawley rats; 6-8 weeks old, weighing approximately 250-300g. The reverse U-Shaped bipedicled flap models based on superficial inferior epigastric (SIE) vessels was used. The reverse U-shaped skin flap was designed to receive half of the blood flow from the SIE vessels on each side, and thus, an ischemia and congestion model could be easily established by ligating the SIE artery and SIE vein on one side.

After anesthesia via an intramuscular injection of a mixture containing 100 mg/kg of ketamine and 10 mg/kg of rumpun, the surgical procedure for establishing the flap model was performed wearing a Loupe. To expose the ventral skin of the rat, the hair on the abdomen was removed with a hair-removal cream. After placing the rat in the supine position, all four legs were fixed and the reverse U-shaped flap was designed (Fig. 1A). The flap was designed to use the midpoint of the imaginary line connecting the xiphoid and pubis as the top of the reverse U shape, with a width of 1 cm. After skin incision with a

blade, microscissors were used to perform the dissection superficially above the anterior abdominal wall fascia, which allowed the feeding vessels to be easily reached from below. It was confirmed that the femoral vessels ran downward towards the leg, while the SIE vessels ran upwards towards the skin flap (Fig. 1B). Since the arteries were located on the lateral side, SIE artery were ligated with vicryl 5-0 (Fig. 1C). The right pedicle was not ligated (control side), meaning only the left pedicle was ligated (ischemic side). Before covering the skin flap, a polyurethane film was placed underneath the skin to minimize the influence of collateral vessels and nylon 5-0 was used to suture the skin. To prevent infection in the surgical site, betadine was used to sterilize the surgical site every day while monitoring it. The present study examined the differences in blood flow changes in the ligated and non-ligated sides from a single rat.

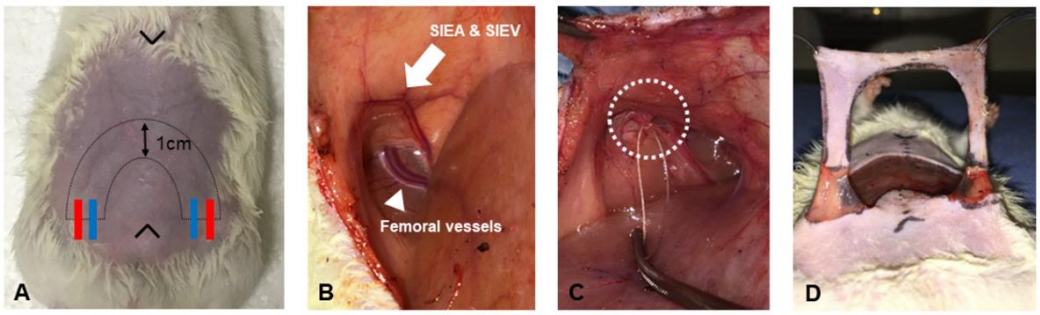

Figure 1. Reverse U-shaped abdominal fasciocutaneous bipedicled skin flap model. (A) Landmarks and incision line (black dot). Red lines indicate artery and blue lines indicate vein which supply abdominal skin. (B) Superficial inferior epigastric artery (SIEA) and superficial inferior epigastric vein (SIEV) ran upwards towards the skin flap (white arrow). Femoral vessels ran downward towards the leg (white arrowhead). (C) To generate ischemic skin flap, ligation of SIEA was performed on one side using vicryl 5-0 (white circle dot). (D) Skin flap was fully dettached and a polyurethane film was inserted underneath the skin and skin was sutured using nylon 5-0.

2. Establishment of murine random skin flap model

This study was approved by the Institutional Animal Care and Use Committee of the Yonsei University Wonju College of Medicine (Protocol YWC-190425-1). Seven-week-old male Balb-c/nu mice (Jackson Laboratories, Bar Harbor, ME) were acclimatized for 7 days and maintained in an air-conditioned specific-pathogen-free room at 21°C with a light/dark cycle of 12 hours/12 hours. To induce anesthesia, pentobarbital sodium (PBS) (20 mg/kg body weight) was intraperitoneally injected into nude mice. A 2 cm × 4 cm rectangular flap was designed on the dorsal skin of the mice (Fig. 2A). A caudally based flap elevation, including the elevation of the panniculus carnosus muscle, was performed (Fig. 2B). A polyurethane film was inserted between the flap and dorsal muscle to stop the blood supply, and the skin was sutured with nylon 5-0 sutures. PDRN (8 mg/kg) was subdermally injected at 12 different points across the proximal, middle, and distal parts of the flap in mice in the experimental group (n=10). In the control group (n=10), PBS was injected at the same locations and at the same concentration as PDRN. The solutions were injected immediately postoperatively.

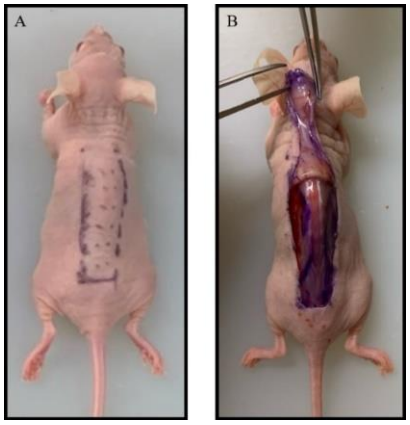

Figure 2. Photographs of random skin flap model. (A) Rectangular random skin flap on the dorsal side of the mouse. (B) A caudally based flap elevation was performed and a polyurethane film was inserted under the skin flap.

3. Laser speckle contrast imaging modality

The LSCI modality used in this study is not commercially available but was developed at

biomedical optics laboratory at Department of Biomedical Engineering, Yonsei University, Korea. It consists of a light source unit : near-infrared (NIR) laser diode (HL8338MG, Thorlabs, USA), laser diode mount (TCLDM9, Thorlabs, USA), laser diode driver (IP500, Thorlabs, USA), laser diode temperature control (3W Temperature Controller, Thorlabs, USA) and xenon lamp (Xenon Nova 330, Karl Storz Endoscope, Tuttlingen, Germany); an image acquisition unit : a charge-coupled device (CCD) camera (Manta G-145B NIR, Allied Vision Technology, Nederland), imaging lens (16mm/F1.4 VIS-NIR, Edmund optics, USA); an image processing unit : CPU (I7-8750H, Intel, USA), GPU (GTX1050Ti, Nvidia, USA). The light source unit has dual light sources of xenon lamp for white light imaging and NIR laser diode for blood flow imaging and can be interchangeable using the converter (Fig. 3). The laser speckle images acquired by NIR CCD camera were processed by using a spatial analysis algorithm with 5x5 window and the vascular flow was monitored by a graphical user interface (GUI) program built with a MATLAB (MatlabR2017a, Mathworks, USA). A previous LSCI modality was partially modified for the specific use of this study.[11)]

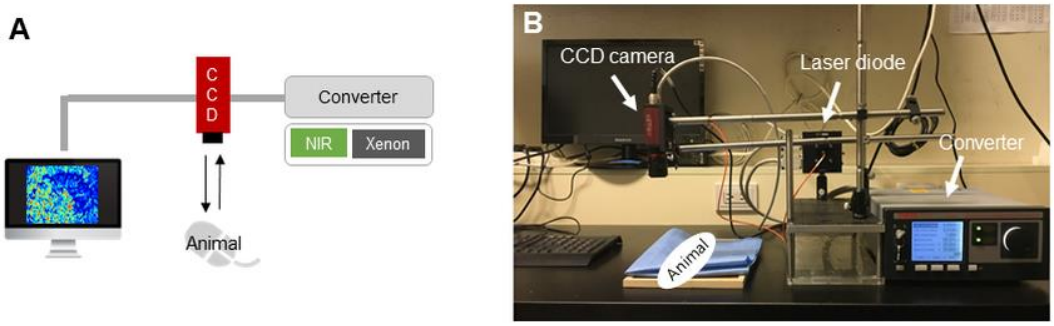

Figure 3. Laser speckle contrast imaging modality setup. (A) A schematic of imaging modality. (B) Real imaging devices. (NIR : near infrared, CCD : charge-coupled device)

4. Laser speckle contrast image analysis method

The working distance between the camera and animal was maintained at 25-30 cm. The

camera was focused on the skin flap region of rat ventral side. Images were sequentially taken first in the white light for color image and then, in NIR for laser speckle image that finally is processed to LSCI of 8 frames/s. Total 40 sequential frames were acquired and averaged for noise reduction (Fig. 4). In the acquired images, regions of interest (ROI) of the skin flap were used to establish a rectangle. Subsequently, the blood flow variation among these regions were compared and were converted into blood flow index (BFI) as arbitrary units (AU) to provide quantitative information of blood flow.[12)]

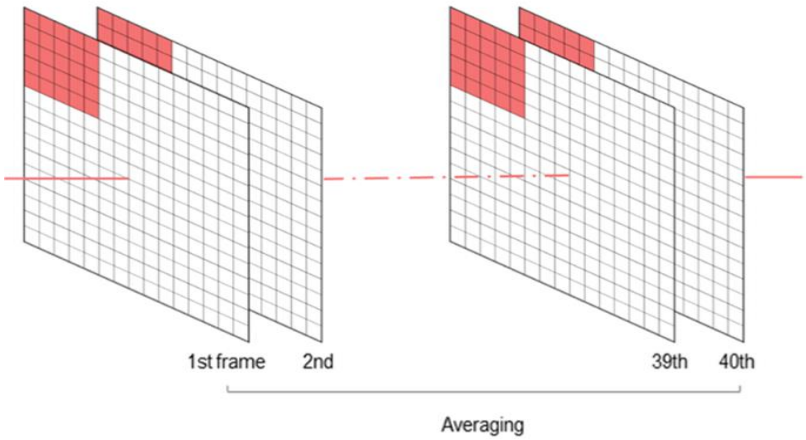

Figure 4. Laser speckle images and image analysis method. Schematic diagram of the laser speckle contrast images averaging analysis.

5. Acquisition of laser speckle images in murine bipedicled skin flap model

The color changes of skin flap were evaluated by naked eyes and the LSCI modality 6, 24, and 48 hours after surgery and postoperatively. The working distance between the camera and animal was maintained at 25-30 cm. The camera was focused on the skin flap region of rat ventral side. Images were sequentially taken first in the white light for color image and then, in NIR for laser speckle image that finally is processed to LSCI. In the acquired images, 3 regions of interest (ROI) on the left and right sides relative to the center of the skin flap were used to establish a rectangle. The ROI was selected at 6 regions; 1-6 starting

from the bottom-right and with enough space in between (Fig. 5). Subsequently, the blood flow variation among these regions were compared and were converted into blood flow index (BFI) as arbitrary units (AU) to provide quantitative information of blood flow.

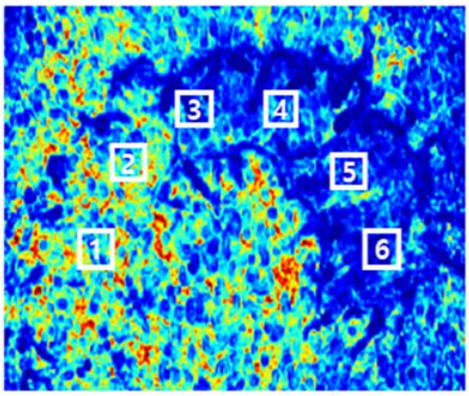

Figure 5. Region of interest (ROI) in laser speckle images. ROI was established as 6 regions.

6. Acquisition of laser speckle images in murine random skin flap model

The surgical sites were examined each day grossly to determine the degree of skin necrosis. On postoperative day 7, the mice were anesthetized using inhalation anesthesia, and images of the flaps were obtained. The Image J software program (National Institute of Health, Rockville, MD) was used to determine the percentage of the survived tissue area relative to the total flap area. A laser speckle contrast imaging (LSCI) device developed by Jung's group was used to quantitatively analyze flap perfusion.[13] The flaps were divided into proximal, middle, and distal parts, and the ratio of the perfusion signal of the distal part to the perfusion signal of the normal skin was compared. A perfusion signal of 1 was assigned to normal skin.

7. Histologic examination in murine bipedicled skin flap model

Tissue samples were harvested from each flap region (region 1 and 6) after sacrifice on

POD 2. The tissues were fixed in 4% paraformaldehyde (Biosesang, Seongnam, Korea) for 24 hours and paraffin-embedded in paraffin wax. Serial sections (4μm thick) were mounted on glass slides and stained with hematoxylin and eosin.

8. Histologic examination in murine random skin flap model

The mice were euthanized on postoperative day 7, and tissue was collected from the surgical site. The collected tissue was fixed in 10% formalin for 24 hours, washed in X-wash for 12 hours, and dehydrated in an automatic tissue processor for clearing. The tissue was embedded in paraffin and sectioned into 4-μm-thick blocks. Tissue slices were deparaffinized with xylene, rehydrated with ethanol, treated with hydrogen peroxide methanol for 10 minutes to block endogenous peroxidase, and washed with distilled water followed by 50 mM Tris Buffer. Subsequently, the samples were treated with goat serum for 30 minutes. After the remanent solution was removed from the slides, they were treated with vascular endothelial growth factor α (VEGFα; Affinity, Changzhou, China) and IL-1β (Affinity, Changzhou, China) primary antibodies at 4℃ for 12 hours. Thereafter, the slides were washed three times with Tween/Tris-buffered saline (TTBS), treated with secondary antibodies (which were the same as the primary antibodies) for 30 minutes, and washed three times with TTBS for 5 minutes. The slides were developed using ImmPACT DAB substrate kit (Vector, California, USA) for 1 minute and washed with distilled water for 5 minutes. Following the wash, the slides were stained with hematoxylin and washed with distilled water. Then, the slides were treated with 1% acid alcohol once or twice, washed for 5 minutes, treated with 1% ammonia water, and washed for 3 minutes. The slides were dehydrated with 70%–100% alcohol for 1 minute at each alcohol concentration, treated with xylene for 3–5 minutes, and mounted on a cover glass. Once staining was complete, the slides were analyzed using a Slide Scanner (Motic, San Francisco, USA). Solution for Automatic Bio-Image Analysis (SABIA) software (EBIOGEN, Seoul, Korea) was used to quantitatively analyze the stained tissues. IL-1β expression was analyzed to examine the differences in inflammatory responses in flaps between the two groups. The level of IL-1β

expression was compared by measuring the area of the IL-1β-positive epidermis in the stained tissue. VEGFα expression was analyzed to investigate the mechanisms of necrosis protection provided by PDRN. The level of VEGFα expression was determined by counting the cells deemed VEGFα-positive per unit area of the epidermis in the stained tissue.

9. Statistical analysis

A two-way analysis of variance test was used to compare the wounded areas between the groups. The Kruskal–Wallis test was used to analyze the results of the histological examinations. We performed analyses of variance to compare the BFI and Kruskal-Wallis tests to compare the BFI between mice. Statistical analysis was performed using the R software (R Foundation, Basel, Switzerland) and the pROC package. The Youden index method was used to calculate the optimal cut-off point.

III. RESULTS

1. Laser speckle image changes in murine bipedicled skin flap model

Figure 6 illustrates the skin flap photographs (Fig.6A), laser speckle contrast images (Fig.6B) and the averaged BFI values in selected ROIs (Fig.6C). Necrosis starts from the region 6 closest to the ligated pedicle and spreads to region 4-5. Full thickness skin necrosis in the vessel ligated region appeared within 2 days after surgery. However, skin necrosis was absent in non-ligated region.

In the murine flap model, the skin color in region 5-6 began to change to a bluish color compared to region 1-2 immediately postoperatively. In the LSCI pseudo color maps, BFI significantly decreased slightly in region 5-6 compare to region 1-2. After surgery, significant decrease of BFI in region 3-4 were found. Region 3-4 were distal part of the flap far from both pedicles. At 6 hours postoperatively, the skin color changes in region 6 got worse. In the LSCI pseudo color maps, BFI recovery in region 3-4 was found and region 6 had lowest BFI. At 24 hours postoperatively, region 5 also began to change its color, and skin necrosis was detectable in region 6 by the naked eye. There was a significant decrease

of BFI in region 5-6 compared with region 1-2. At 48 hours postoperatively, total necrosis occurred in region 4–6. BFI dramatically decreases in region 6 as necrosis progressed. BFI of region 1-2 is significantly higher than that of region 5-6. The region where the BFI decreases coincides with the region where skin necrosis occurs. There were statistically significant differences between the region 1 and 6 over time after surgery. Figure 6 shows that post-hoc analyses revealed statistically significant differences between groups (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

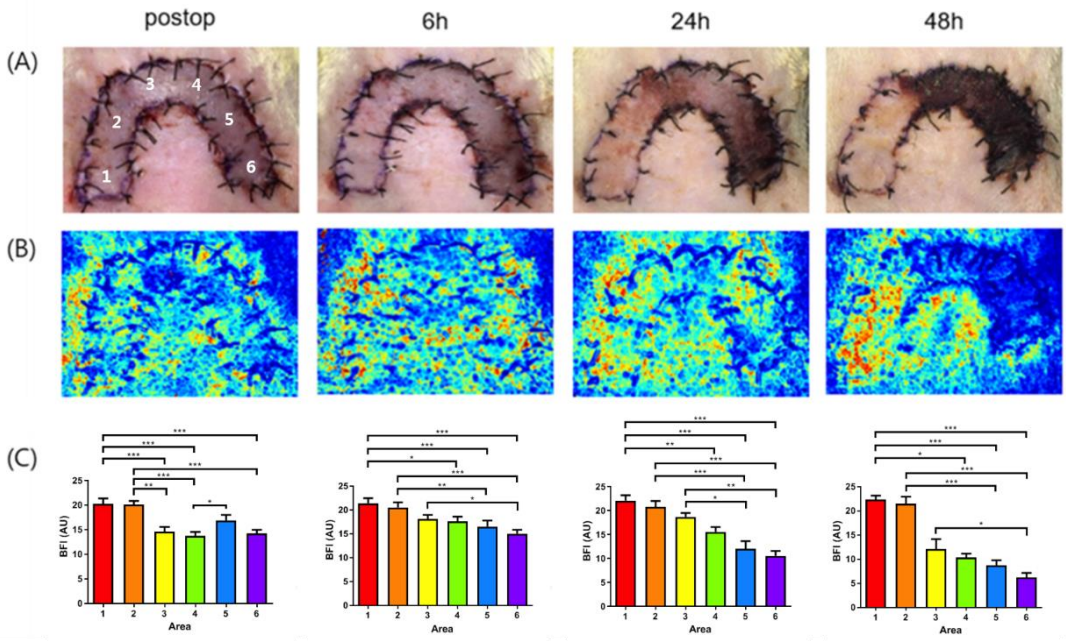

Figure 6. Laser speckle image analysis in bipedicled skin flap model. (A) The serial photographs of ischemic skin flap. Flap necrosis started on the postoperative day (POD) 1 and was completed on the POD 2. (B) Laser speckle contrast images of skin flap. Blood flow index (BFI) was decreased in vessel ligated region indicating a reduction of the blood flow. The color scale represents blood flow changes from high (red) to low perfusion (blue). (C) The average BFI in the selected regions. The perfusion of skin flap was reported in arbituary units (AU). Values correspond to mean ± SEM. There were statistically

significant differences between groups (*p < 0.05, ** p < 0.01, *** p < 0.001).

2. Histologic analysis of flap in murine bipedicled skin flap model

Skin samples obtained in region 6 (ischemic side) (Fig. 7B) were histologically different from samples obtained in region 1 (control side) (Fig. 7A). H&E staining presented subcutaneous edema and extravascular erythrocytes in the dermis in region 6. Red blood cell deposit and inflammatory infiltrate could were observed in the panniculus carnosis among ischemic region. Histological analysis clearly presented that full-thickness skin necrosis were induced in the ischemic region of skin flap. The region 1 exhibited less necrosis than region 6 by H&E staining.

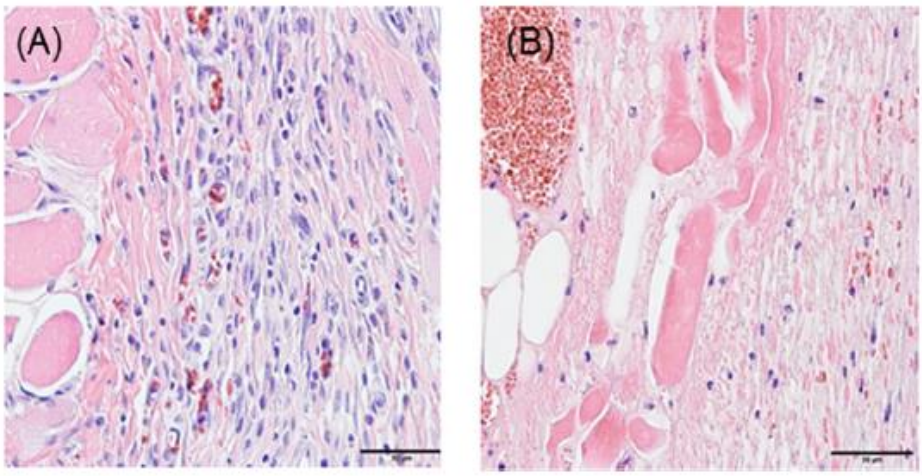

Figure 7. Histologic analysis by H&E staining. The region 1 (A) and region 6 (B) of the skin flap on the 2 days after the surgery (magnification, ×10; scale bar: 200 μm).

3. Cut off value of laser speckle images in murine bipedicled skin flap model

The cut-off value was determined based on the BFI confirming the presence or absence of tissue necrosis. Receiver Operating Characteristic (ROC) curve analysis showed that when the BFI was under 12.5, the flap necrosis rate was significantly increased (area under the

curve = 0.991, sensitivity = 96.0%, specificity = 98.9%). The asymptotic p-value is less than 0.05, and the lower bound and upper bound of the asymptotic 95% confidence interval are both greater than 0.5. The results are shown in Figure 8.

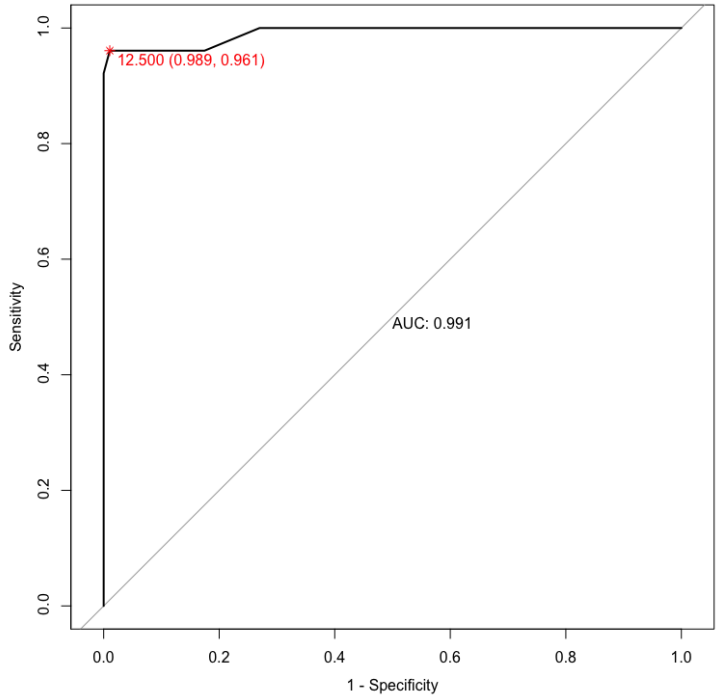

Figure 8. Receiver operating characteristic (ROC) curve analysis of laser speckle images in bipedicled skin flap model. It shows the diagnostic performance of the laser speckle imaging for prediction of flap necrosis in murine flap model.

4. Skin flap viability in murine random skin flap model

All 20 mice survived until postoperative day 7 with no flap loss due to infection or cannibalism. Postoperative flap necrosis began at the distal end of the graft. On postoperative day 7, the percentage of surviving flap tissue area relative to the total flap area was significantly higher in the experimental group (60.87%±7.63%) than in the control group (45.23%±10.72%) ($p<0.05$) (Fig. 9A, 9D). In the control group, necrosis was

observed along the inner surface of the flaps, as was swelling and blood congestion (Fig. 9F). No significant skin discoloration was observed in the experimental group, and the vessels maintained their shape (Fig. 9C). The mean LSCI perfusion signal of the distal part of the skin flap in the control group was 0.57±0.12, and that in the experimental group was 0.74±0.13 ($p<0.05$) (Figs. 9B, 9E and 10).

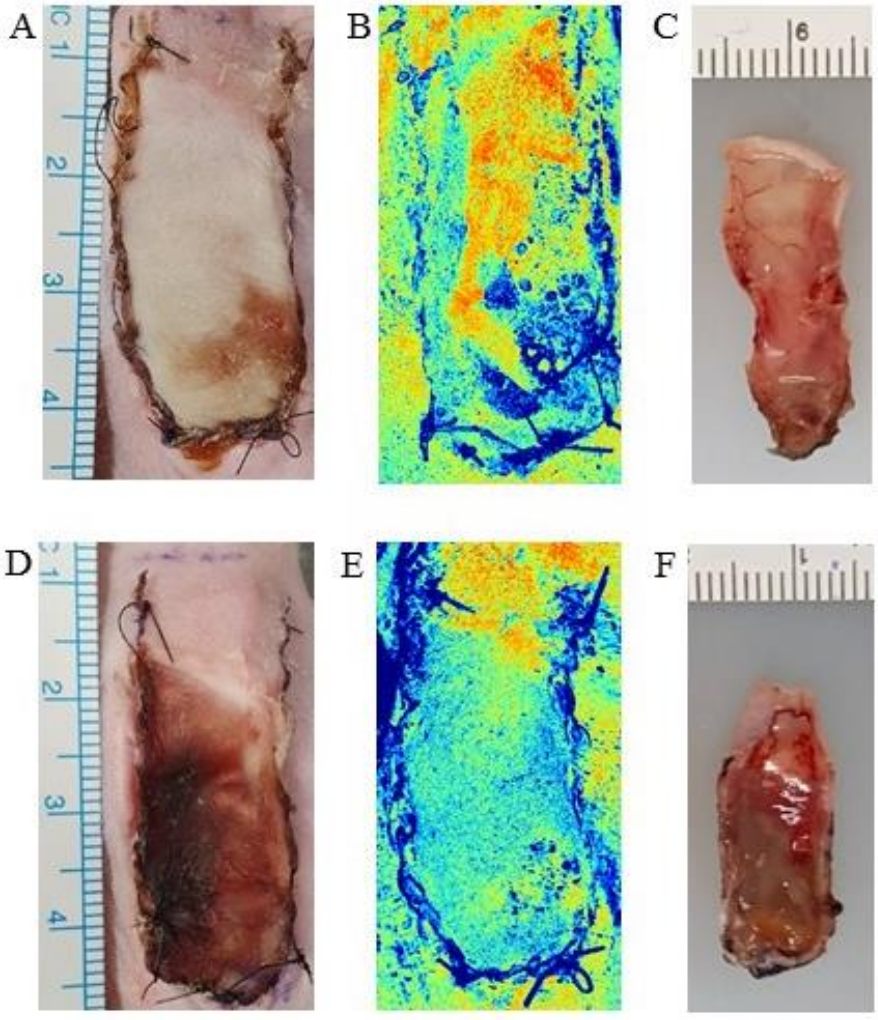

Figure 9. Photographs on postoperative day 7 in random skin flap model. (A) Photograph from the polydeoxyribonucleotide (PDRN) group. (B) Laser speckle contrast image from

the PDRN group. (C) Inner surface of skin tissue from the PDRN group. (E) Photograph from the pentobarbital sodium (PBS) group. (F) Laser speckle contrast image from the PBS group. (G) Inner surface of skin tissue from the PBS group.

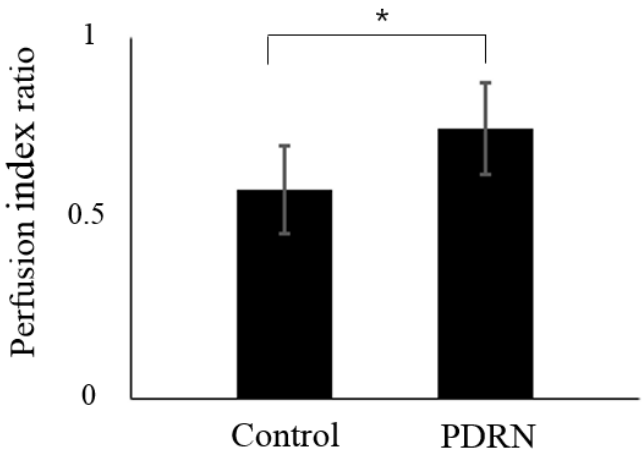

Figure 10. The laser speckle perfusion index ratio in random skin flap model. †Polydeoxyribonucleotide, PDRN (Perfusion index ratio = perfusion signal of distal part / perfusion signal of normal skin) (*$p < 0.05$).

5. Histopathological changes in murine random skin flap model

Histologic analysis revealed skin lacerations resulting from skin necrosis in the control group. Necrosis, marked edema, abscesses, and severe inflammation were observed in the control group skin flaps (Fig. 11A). The skin flaps in the experimental group had normal epidermis and dermis; however, inflammatory cells and fibrosis were observed in the subcutaneous layer (Fig. 11B). Partial formation of granulation tissue and increased formation of capillary vessels were observed in the experimental group.

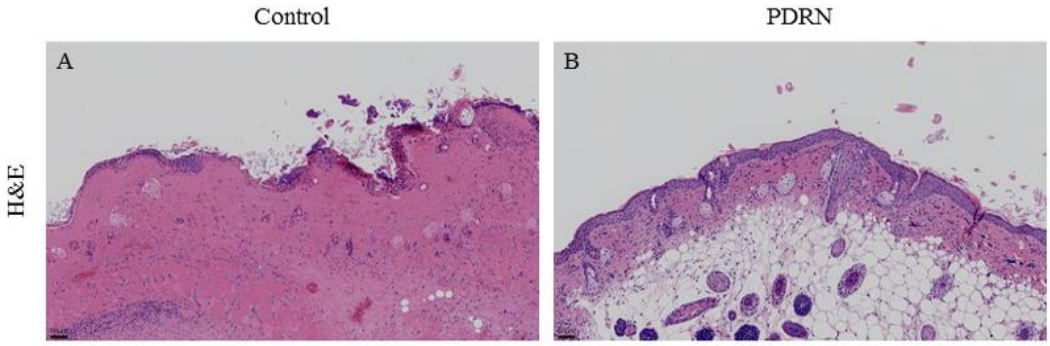

Figure 11. Hematoxylin & eosin (H&E) staining. Pentobarbital sodium (PBS) group (A), Polydeoxyribonucleotide (PDRN) group (B). (Magnification, 20×; scale bar: 200 μm)

6. Expression of IL-1β and VEGFα in murine random skin flap model

Immunohistochemistry (IHC) of IL-1β and VEGFα was performed to identify the reason for the PDRN group's increased skin flap survival (Fig.12). The experimental group (1,286 μm^2/microscopy field at 20× magnification) had significantly lower IL-1β expression than the control group (13,620.33 μm^2/microscopy field at 20× magnification; $p<0.05$) (Fig. 12A,B and 13A). The experimental group had significantly higher VEGFα expression than the control group (506/mm^2 versus 32/mm^2; $p<0.05$) (Fig. 12C,D and 13B).

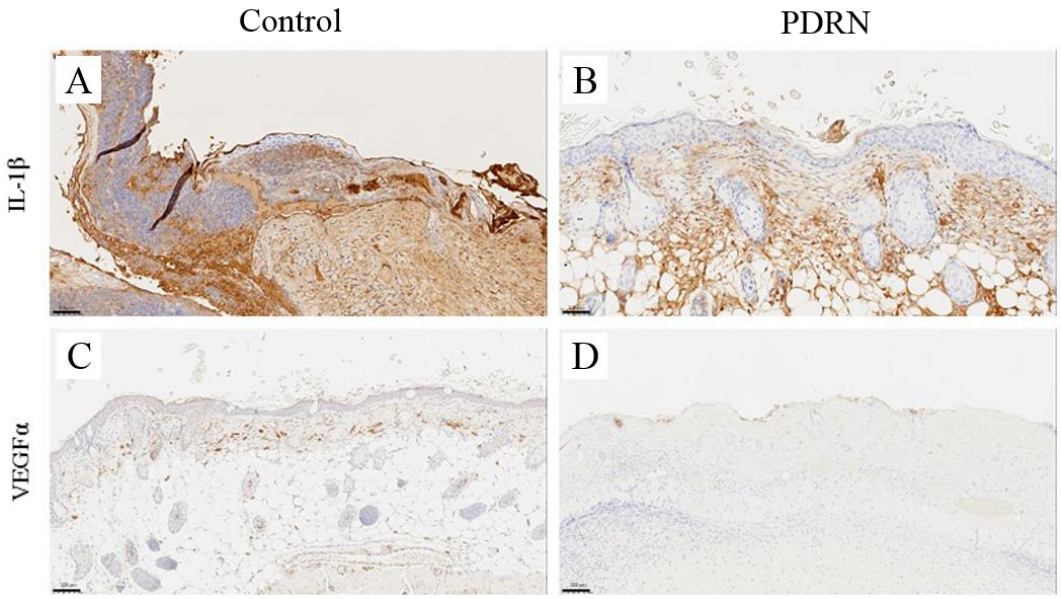

Figure 12. Hematoxylin & eosin (H&E) staining and immunohistochemistry (IHC) analysis. Pentobarbital sodium (PBS) group (A, C, E), Polydeoxyribonucleotide (PDRN) group (B, D, F). (Magnification, 20×; scale bar: 200 μm)

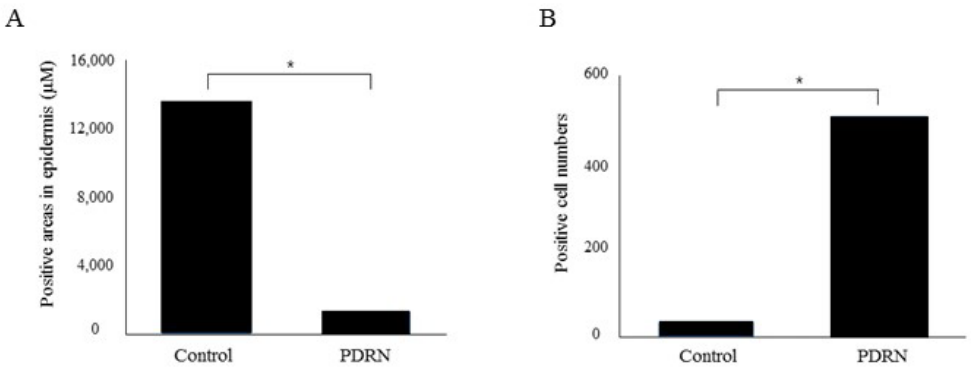

Figure 13. Histograms of optical density. Values of (A) IL-1β and (B) VEGFα (*, $p<0.05$). †PDRN, polydeoxyribonucleotide.

IV. DISCUSSION

In spite of notable advancements in free flap reconstruction techniques, the development of postoperative flap monitoring devices and protection against ischemia-reperfusion injury has been progressing slowly. In clinical practice, handheld Doppler devices are commonly employed due to their portability. Nevertheless, obtaining precise measurements can be challenging for inexperienced examiners, as these devices have a limited measurement scope and discerning normal blood flow from vascular anastomosis site blood flow by sound alone is a complex task. Additionally, several drugs have been used post-flap surgery in an attempt to prevent distal flap necrosis, but their efficacy has been limited. Our study suggests that a flap monitoring system utilizing laser speckle images to detect flap necrosis post-surgery can serve as an early diagnostic tool for identifying flap necrosis. Furthermore, our research confirmed the effectiveness of injecting PDRN into the flap for protecting against flap injury. Simultaneously employing both methods could be a promising strategy for preventing flap necrosis.

Laser speckle refers to an irregular destructive and constructive interference pattern happening when laser is reflected or scattered on an object. When an object moves, the phase of the scattered constructive interference changes, thereby altering the speckle pattern. An analysis of the changing speckle pattern provides information regarding the object's movement.[14] When the speed of red blood cells in the blood vessels increases, the fluctuation of the speckle pattern also increases, causing a blurring in the image. Quantifying and visualizing the blurring then produces intuitive blood flow information.[15] Thus, LSCI enables non-invasive real-time monitoring of cutaneous blood perfusion in a large skin region.[16] The study utilized an LSCI system to examine changes in blood flow within the skin flaps, with results consistent with the gross anatomical findings. The experimental group of mice injected with PDRN showed a higher perfusion index in the distal part of the flap and better blood flow preservation compared to the control group. This highlights the potential clinical feasibility of LSCI as a skin flap monitoring device, providing real-time, non-invasive information on cutaneous blood perfusion. Furthermore,

we endeavored to establish a predictive threshold for flap necrosis by deriving the cutoff value from the LSCI-derived BFI in a murine bipedicled skin flap model. When the BFI reached a threshold of 12.5, the area under the curve was calculated to be 0.991, with a sensitivity of 96.0% and specificity of 98.9%. We propose that a BFI below 12.5 should be considered the critical threshold value, as indices falling below this value are indicative of an increased risk of necrosis. This threshold, as determined in our experimental setting, has yet to demonstrate its validity in the clinical application of this method in patients undergoing procedures involving flaps. However, we firmly consider a BFI of 12.5 as a reliable cut-off value. Indices falling below this threshold should warrant a surgical evaluation to either enhance flap perfusion, if feasible, or consider early salvage flap surgery. The need for further research and clinical validation to establish the applicability of this threshold in diverse clinical scenarios is evident.

The results of this study demonstrate that subdermally injected PDRN effectively enhances the survival of random skin flaps by promoting angiogenesis and reducing inflammation. The PDRN group showed minimal histopathological changes during flap necrosis, along with lower IL-1β expression and higher VEGFα expression in immunohistochemistry staining. These findings suggest the potential therapeutic role of PDRN for random skin flap necrosis.

PDRN is a deoxyribonucleotide mixture extracted from salmon trout or chum salmon semen DNA, processed through purification and sterilization to remove immunogenic proteins and peptides. In vivo studies have revealed that the main mechanism of PDRN is the activation of the adenosine A2A receptor that plays a central role in regulating inflammation, ischemia, and angiogenesis[10]. Previous studies have reported the protective effects of intraperitoneally injected PDRN and subdermally injected PDRN in rat skin flap models before and after surgery.[17,18] In contrast, this study focuses on the protective effects of a single, local injection of PDRN immediately after surgery, providing valuable insights into its potential clinical application. After PDRN injection, the VEGFα level was increased, and the IL-1β level was decreased compared to the levels in animals injected with PBS.

VEGF plays a crucial role in wound healing by promoting angiogenesis. It increases vascular permeability and degrades the extracellular matrix (ECM) to facilitate endothelial cell migration and proliferation and prevent endothelial cell apoptosis.[19] In ischemic tissues, the level of VEGF in endothelial cells increases, angiogenesis is promoted, and mediators are supplied through capillaries to promote healing.[20] Angiogenesis promoted by increased VEGF induces granulation tissue formation and maturation, elastic fiber formation, and rapid healing to protect the tissue from necrosis.[21,22] In this study, PDRN was locally injected into the skin flaps, and VEGF expression was analyzed using IHC 7 days later. Increased VEGF expression was observed after PDRN injection, especially around new vessels. Moreover, an increased number of VEGF-positive cells per microscopic field was noted in the mice injected with PDRN. These results suggest that PDRN increases VEGF expression during the conditions of skin necrosis to promote angiogenesis, thereby contributing to wound healing.

IL-1β is a key pro-inflammatory cytokine that regulates inflammation and immune responses to infections. IL-1 consists of IL-1α and IL-1β, which are expressed by different genes.[23] IL-1β is a major pro-inflammatory interleukin. When IL-1β-driven inflammatory signals are overexpressed at a wound site, inflammatory cells stay at the site longer, and the level of matrix metalloproteinases that degrade the ECM increases, delaying wound healing.[24] Blocking IL-1β expression is considered an effective strategy to reduce inflammation and promote wound healing.[25] In this study, a decreased expression of IL-1β was observed in the experimental group 7 days postoperatively, suggesting that PDRN inhibited inflammatory reactions by reducing IL-1β expression, resulting in protection of the skin from necrosis.

Preclinical studies have reported that PDRN has tissue regenerative and anti-ischemic properties. PDRN has been shown to restore damaged skin and promote wound healing by increased VEGF expression in diabetic mice.[26] Furthermore, PDRN improved circulation in an animal model of peripheral arterial occlusive disease through increased VEGF expression.[27] PDRN treatment demonstrates significant effectiveness in reducing the

secretion of pro-inflammatory cytokines. Notably, inflammation serves as a compensatory response to cellular and tissue damage triggered by ischemia-reperfusion injury. It's noteworthy that the induction of ischemic damage initiates the activation of the MAPK cascade, orchestrating a wide array of cellular processes. PDRN treatment proves to be efficacious in mitigating inflammation by attenuating phosphorylation within the MAPK cascade pathway.[28] PDRN exhibits remarkable potential as an A2A receptor agonist. Through A2A receptor activation, PDRN potentially inhibits the NF-κB pathway while concurrently enhancing the Wnt/β-catenin signaling pathway. Activation of the A2A receptor leads to an elevation in cyclic adenosine monophosphate (cAMP) levels, consequently upregulating the Wnt signaling pathway. This upregulation facilitates the accumulation of β-catenin within the cytoplasm, promoting its translocation into the nucleus and thereby fostering gene transcription. The activation of the Wnt/β-catenin pathway exerts particular influence over immune-inflammatory mediators (e.g., IL-1β) and genes crucial for tissue healing (e.g., VEGF).[29] There exists a compelling rationale for further exploring PDRN's clinical potential across a spectrum of pathological conditions. Combined with the results of this study, these previous findings suggest that PDRN may be effective in clinical settings for the restoration of damaged flaps and improved flap survival. Future studies regarding the mechanism of purinergic signaling are warranted.

Despite the promising results, there are challenges in applying these findings to clinical practice. One strength of LSCI is that it enables a wide-field monitoring of the entire flap site, thereby allowing examiners to monitor both pedicles and surrounding blood flow in real time. In contrast, there are still several limitations in the clinical use of this setting. First, LSCI cannot be used to obtain images deep in the skin. In the present experiment, we used a laser diode with a wavelength of 830 nm NIR light. NIR light enables penetration up to 1 cm beneath the skin. In rats, the depth from the skin to the pedicle is relatively small; hence, adequate assessment was possible using LSCI. In contrast, humans have a deeper depth from the skin to the pedicle; hence, observing blood flow changes in buried flaps is difficult, such as in pharyngolaryngeal reconstruction, using LSCI. However, blood flow

around the pedicle could be adequately observed for free flaps around the head and neck area using NIR-based LSCI. Second, this device is relatively large and has poor portability; hence, assessment of the flaps located in the oral cavity is difficult. The camera device should be downsized with better flexibility to enable assessment of more diverse types of flaps. Third, LSCI is still semiquantitative measurement. LSCI cannot measure the absolute blood flow rate. Instead, the measurement results are presented as a relative index known as AU. Because the values can vary depending on the measurement interval setting, it can be used to determine the difference from the normal tissues. Fourth, Large-animal experiments and clinical trials must be carried out for clinical use, as this study was only applied to small animals. Additionally, when PDRN is injected into the human body, it is crucial to evaluate the appropriate injection dose, injection route, and injection timing. Although the results after 1 week were confirmed in this study, additional studies are required on the long-term efficacy and side effects of PDRN. Further, to confirm the exact mechanism of action of PDRN, studies on various biomarkers should be performed.

V. CONCLUSION

This study provides valuable evidence supporting the potential therapeutic role of PDRN and LSCI in enhancing the survival of skin flaps. The combination of PDRN and LSCI offers promising avenues for clinical applications. However, further research, including large-animal studies, clinical trials, and investigations into the underlying mechanisms, is essential to fully comprehend the clinical potential and challenges of using these strategies in reconstructive flap surgery.

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ABSTRACT(IN KOREAN)

피판 수술 후 피판의 보호 전략:
실시간 레이저 스펙클 기반 혈류 모니터링을 통한 혈류 이상 탐색 및 PDRN 주입을 통한 피판 보호 효과

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본 연구는 피판 수술 후 실시간으로 레이저 스펙클 영상 장비를 활용하여 피판의 혈류 모니터링을 통해 혈류 이상을 조기에 진단하고, 피판의 신생혈관형성을 돕는 PDRN 약물을 피판에 주입하여 피판을 허혈성 괴사로부터 보호할 수 있는 이중 전략을 제시하고자 한다.

양측 유경 피부 피판 동물 모델에서 혈관 결찰군과 정상 피판군을 구축한 뒤 육안 소견과 레이저 스펙클 영상을 비교하여 피판 괴사 정도와 레이저 스펙클 영상 소견의 연관성을 확인하였다. ROC 곡선에서 blood flow index 를 12.5로 cut off value를 설정하였을 때 피판 괴사 진단률이 민감도 96%, 특이도 98.8%로 피판 괴사 예측을 위한 레이저 스펙클 영상의 진단 성능을 보여주었다.

임의 피부 피판 동물 모델에서는 PDRN 약물을 주입한 군과 PBS를 주입한 군의 피판 괴사 정도를 비교하였다. 생존한 피판의 면적 비율은 PDRN 군이 PBS군 보다 통계학적으로 유의하게 높았다. 또한, PDRN군은 PBS군에 비해 IL-1β 발현이 통계학적으로 유의하게 낮았고, VEGFα의 발현이 통계학적으로 유의하게 높았다.

본 연구 결과는 동물의 피부 피판 모델에서 피하 주사된 PDRN이 피판의 괴사가 일어나는 동안 피판 생존을 향상시키는 데 효과적임을 시사하며, 레이저 스페클 영상 시스템이 피판 관류의 이상을 조기에 진단할 수 있는 영상장비로서 유용하게 사용될 수 있는 기초 자료를 제공하였다.

핵심되는 말 : polydeoxyribonucleotides; surgical flaps; laser speckle contrast imaging

PUBLICATION LIST

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