



Effect of Polydeoxyribonucleotide on Chondrocutaneous Composite Grafts Survival

Jae Won Heo¹ · Yong Hun Kim¹ · Eon Su Kim¹ · Sug Won Kim¹ · Jiye Kim¹ 



Received: 14 February 2019 / Accepted: 7 May 2019 / Published online: 21 May 2019
© Springer Science+Business Media, LLC, part of Springer Nature and International Society of Aesthetic Plastic Surgery 2019

Abstract

Background A composite graft is considered the best choice for facial reconstruction because of proper texture, color, and simple surgical techniques. However, due to revascularization by the bridging phenomenon, it has limitations with unpredictable survival rates and can be applied only to small defects. Polydeoxyribonucleotide (PDRN) plays an important role in multiple vascular processes such as angiogenesis via production of a vascular endothelial growth factor and by providing an anti-inflammatory effect by reducing pro-inflammatory cytokines through the adenosine A2 receptor stimulation. Thus, here, we investigated PDRN as a supportive method to improve survival of composite grafts.

Methods Chondrocutaneous composite grafts were applied to both ears of 20 New Zealand White rabbits. The grafts were then rotated and returned to their positions to prevent the original blood flow from the base of the grafts. On postoperative days 1, 3, 6, 9, and 12, PDRN was injected intradermally into the experimental group (20 ears) and normal saline was injected into the control group (20 ears) to exclude bias of pressure effect. After 12 days, graft survival and cutaneous blood flow were examined under laser speckle contrast imaging.

Results Gross observation indicated that the graft viability in the PDRN group was significantly higher than that in the control group ($p < 0.05$). Through laser speckle contrast

imaging, signal intensity increased from the periphery and progressed centrally with treatment.

Conclusion Our findings suggest that PDRN may increase blood flow around at the base of the graft, restore the perfusion, and improve the survival of the composite grafts.

No Level Assigned This journal requires that authors assign a level of evidence to each article. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors www.springer.com/00266.

Keywords Polydeoxyribonucleotides · Tissue transplantation · Graft survival

Introduction

Numerous traumas, excision of skin tumors, and other causes lead to soft tissue defects on the human body. Numerous attempts to treat these defects through reconstructive surgery and, more recently, systematic methods have been established according to the reconstructive ladder: secondary healing, primary closure, skin graft, local, and regional flap to free flap [1]. Careful consideration in choosing the plan for reconstruction should be made to functionally and aesthetically maintain the characteristics of the original tissue. In this respect, a composite graft is a very attractive option for soft tissue defects of the face, because of minimal secondary contraction, proper texture, better color matching with the surrounding skin, and more resistance to contractile forces than other types of grafts [2]. Additionally, when using subcutaneous fat tissue with osteocutaneous or chondrocutaneous composite tissue, it is possible to reconstruct various defects such as three-

✉ Jiye Kim
gen80@yonsei.ac.kr

¹ Department of Plastic and Reconstructive Surgery, Wonju Severance Christian Hospital, Wonju College of Medicine, Yonsei University, 20, Ilsan-ro, Wonju-si, Gangwon-do, Republic of Korea

dimensional structures of the facial area such as the nasal alar and columellar, fingertip injuries, and cartilage–perichondrium composite graft in tympanoplasty [3–5].

However, there are currently some limitations in using such composite grafts. Mostly, composite grafts are limited by their size and survival rates are unpredictable. In addition, graft size should not exceed 1.5 cm in diameter [4], as it would be difficult to predict the survival rate of larger grafts, and ischemia–reperfusion injuries may lead to graft failure. Therefore, several adjunctive therapies have been carried out to increase the survival rate of composite grafts, including hyperbaric oxygen therapy, topical application of nitroglycerin ointment, and intravenous treatment with prostaglandin E1; however, their efficacy is questionable [6–8]. There is little evidence of approaches based on topical injection, which is presumed to be capable to induce angiogenesis directly in the grafted site. In this study, we focused on this phenomenon by examining effects of intradermal injection of polydeoxyribonucleotide (PDRN). It is known that PDRN acts on adenosine A_{2A} receptors to promote tissue regeneration and wound healing. The local injection therapy of PDRN has already been proven qualitatively and quantitatively [9]. After binding to the A_{2A} receptor, PDRN induces fibroblast maturation and vascular endothelial growth factor (VEGF) accumulation in the wound [10], which leads to the induction of angiogenesis, promotion of granulation tissue formation, and promotion of wound healing process [11].

Therefore, we hypothesized that PDRN will be effective for the survival of composite grafts. In our study, we investigated the effect of PDRN on the survival rate of composite grafts in a rabbit model. To assess this hypothesis, we attempted to evaluate tissue blood flow through laser speckle contrast imaging for the first time, which can evaluate percutaneous blood flow using the reflection of laser polarized light [12].

Methods

Polydeoxyribonucleotide (PDRN)

PDRN was extracted from the sperm of *O. keta* (Chum salmon), which is known to have a similar function in tissue repair as human placenta PDRN. Immediately after the surgical procedure, details of which are given below, the rabbits in the experimental group received an intradermal injection of PDRN (Rejuvenex[®], PharmaResearch Products, Seongnam, South Korea) at a dosage of 0.75 mg/kg, 1 cc on each ear. The control group received an injection of 0.9% NaCl of the same volume and dosage.

Surgical Procedure

A total of 20 New Zealand White rabbits (weighing 2.5 to 3.5 kg) were used for the experiment [8]. Prior to composite graft transfers, each rabbit was sedated with intramuscular injections of ketamine and 2% Rompun solution. Both ears were cleansed with alcohol. A 2-cm-diameter, circular, chondrocutaneous section of composite tissue was harvested 4 cm from the external meatus of the ear. Each graft was rotated 90 degrees from the base of the graft and set back to the wound bed, which was fixed with 5–0 blue nylon continuous suture to prevent original blood flow from the base of the graft. The operation was carried out on both ears [13]. Then, 1.0 mL of PDRN (Pharmaresearch, Korea), at a dosage of 0.75 mg/kg, was injected evenly into the surrounding skin of the composite graft with a 26-gauge 1-cc syringe on both ears of the animal in the PDRN group, and 1.0 mL of normal saline (0.9% NaCl) was injected around the graft in the same manner in the control group. The wound was treated with the antibiotic ointment, Bactroban (mupirocin, HanAll BioPharma, Korea).

The experimental protocol was approved by the Animal Care and Experiment Committee of Yonsei Wonju University.

Assessment of Composite Graft Survival Rates

On postoperative days 1, 3, 6, 9, and 12, graft survival was grossly examined and photographed. Areas were measured using ImageJ (1.52 version; National Institutes of Health, Bethesda, Md.) on each follow-up day. The survival rate of the graft was determined by the area of necrotic tissue of the whole graft.

Assessment of Microcirculation in Composite Grafts

The grafted site was observed through laser speckle contrast imaging on days 1, 3, 6, 9, and 12 after the operation to observe minute differences in blood flow as time progressed. With laser speckle contrast imaging, areas with high blood flow are seen as relatively warm colors (red, green, and yellow) and those with poor or no blood flow are observed as blue (Fig. 1) [12, 14].

Immunohistochemical Analysis

The rabbits were euthanized on postoperative day 12 for histological examination. The composite tissue grafts were harvested from the identical location. Immunohistochemical staining of specimens was conducted using anti-CD31 antibody. Two blinded observers counted CD31 + vessels

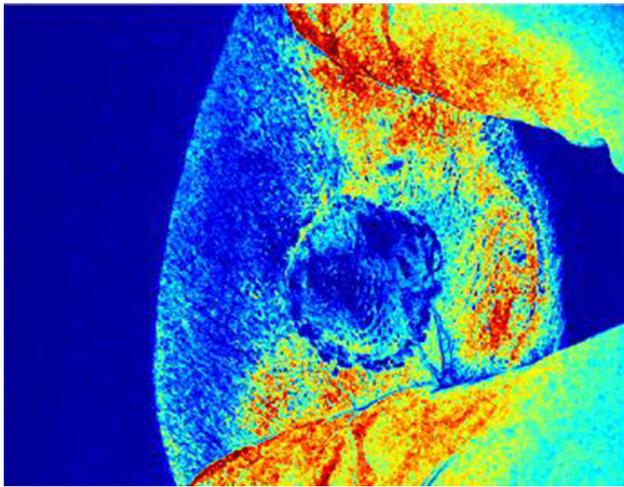


Fig. 1 An example of laser speckle contrast imaging. On the third day after composite grafting, blood flow was not yet observed. The graft was blue and the periphery showed different colors depending on the blood flow

under a microscope (high-power field, 200 ×) to assess the amount of neovascularization.

Statistical Analysis

All results are presented as mean ± SEM. Statistical analysis was performed with a paired *t* test. The level of significance was taken as $p < 0.05$. SPSS V25 software (SPSS Inc, Chicago, IL) was used for statistical analysis.

Results

Effect of Polydeoxyribonucleotide on Composite Graft Survival

When graft survival was examined on postoperative day 12, the necrotic area was observed from the central part of the grafts in both groups. Upon examination, the viable area around the peripheral edge was clearly larger in the PDRN-treated group. On postoperative day 12, necrotic areas were demarcated and survival rates were calculated by measuring the area ratio of the viable area to the total graft area at postoperative day 12. The average viable area in the graft was $85.0 \pm 19.4\%$ in the PDRN group and $69.4 \pm 22.1\%$ in the control group, which was a statistically significant difference ($p < 0.05$) (Fig. 2a). Representative images for each group are shown in Fig. 3.

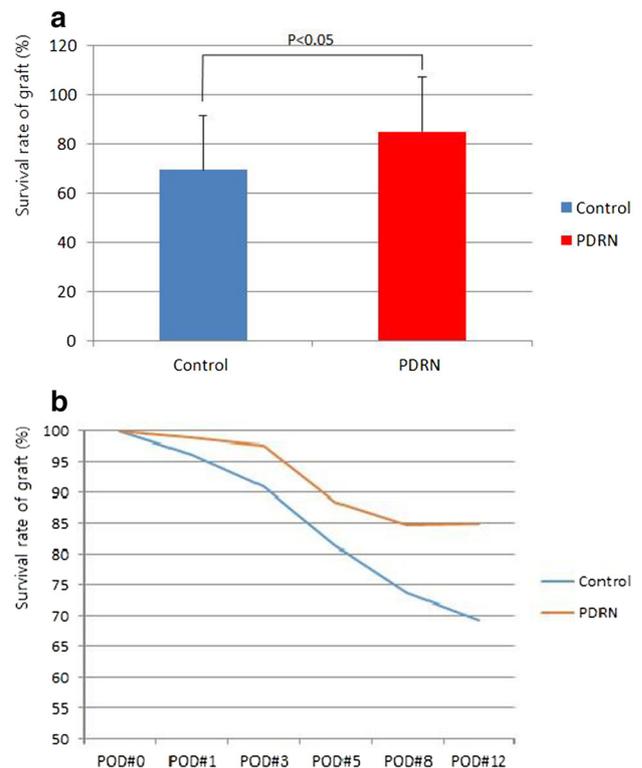


Fig. 2 Effect of polydeoxyribonucleotide on the microcirculation in composite grafts. Survival rate was defined as the ratio of viable portion to total area. Survival rates were significantly higher in the PDRN group when compared to the control group ($p < 0.05$) on postoperative day 12 (Fig. 2a). The line graph shows the trend of survival rate for each follow-up day (Fig. 2b)

Effect of Polydeoxyribonucleotide on the Microcirculation in Composite Grafts

Before the operation, laser speckle contrast imaging was used to assess microcirculation in composite grafts to evaluate original blood flow. Initially, there were no significant differences observed between the groups. Thereafter, measurements were taken immediately after the surgery and on postoperative days 1, 3, 6, 9, and 12. Both groups showed gradual improvement in blood flow over time. There were no significant differences in perfusion between the groups immediately after the surgery. However, when comparing the two groups, we can observe that the laser speckle signal of the PDRN-treated group, i.e., the blood flow, became more prominent over time, especially by postoperative days 9 and 12 (Fig. 4). Notably, in the PDRN group, the blood flow signal initially appeared at the margin of the composite grafts and subsequently spread, occupying most of the graft area by day 12. This was consistent with previous findings that composite graft survival is characterized by initial peripheral neovascularization [3].

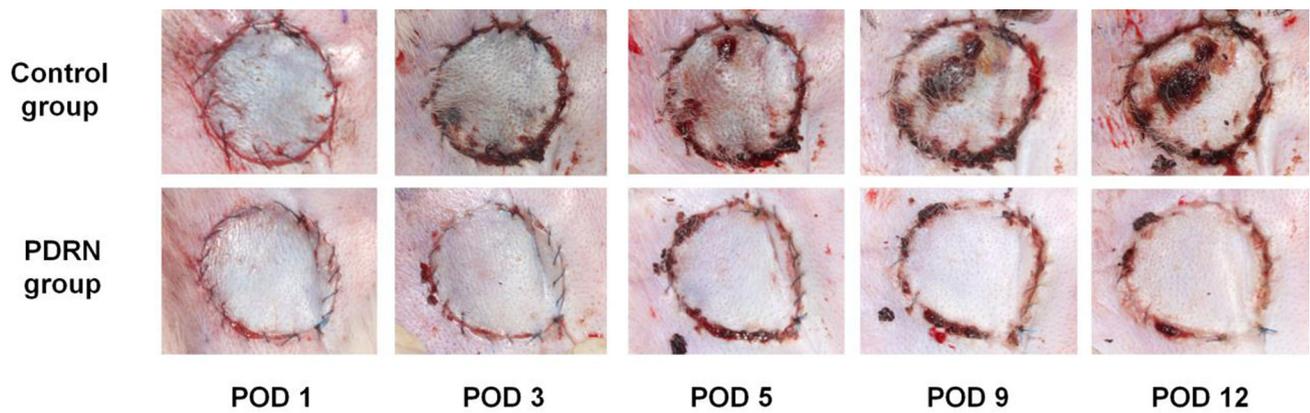


Fig. 3 The representative gross images of the grafts. The representative images of the grafts in the control group (above) and the PDRN group (below). Congestion of the grafts progressed gradually in the

control group and some eschar was formed by postoperative day 5. On the other hand, in the PDRN group, congestion resolved from days 1 to 3 and showed whole graft survival

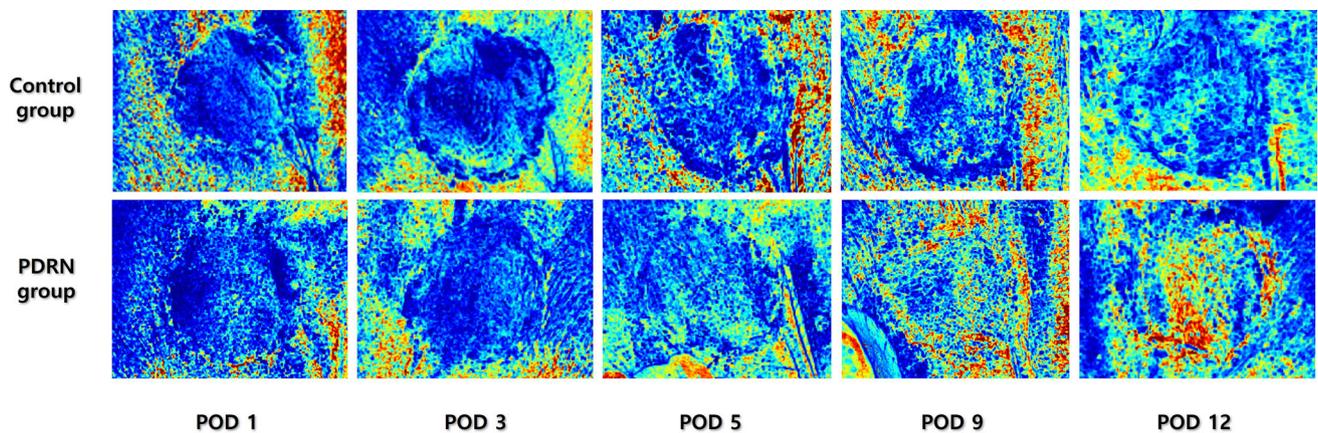


Fig. 4 Effect of polydeoxyribonucleotide on capillary density in composite grafts. The blood flow of the control group and the PDRN group was compared using laser speckle contrast imaging. In the PDRN group, blood flow was slightly increased compared with the

control group 3 days after the operation; in contrast, perfusion in the PDRN group was higher than that in the control group on postoperative days 9 and 12

Effect of Polydeoxyribonucleotide on Capillary Density in Composite Grafts

Immunohistochemical analysis of the specimens on the 12th day using anti-CD31 antibodies revealed clear differences in capillary density between the groups. Representative images of the histology are shown in Fig. 5. The number of capillaries stained with anti-CD31 antibody, which indicates neovascularization, was significantly higher in the PDRN group than that of the control group (control group, 9.7 ± 2.00 ; PDRN group, 14.2 ± 1.99 ; $p < 0.05$; Fig. 6).

Discussion

In this study, we demonstrated that intradermally injected PDRN increased the blood flow to the composite graft, resulting in a significant difference in graft survival rates. Composite grafts showed pale color by ischemia immediately after the operation and followed by a bluish color as venous congestion progressed. During this time, the composite grafts obtain oxygen through ‘plasma imbibition’ before revascularization [15]. After 72 h, neocapillary invasion and revascularization occur, and gradually the graft returns from a pink shade to normal color in 7 days; areas where revascularization was not sufficient show margins of necrosis [16]. Thus, reducing ischemia before the revascularization could be a key for the survival rate of the composite graft.

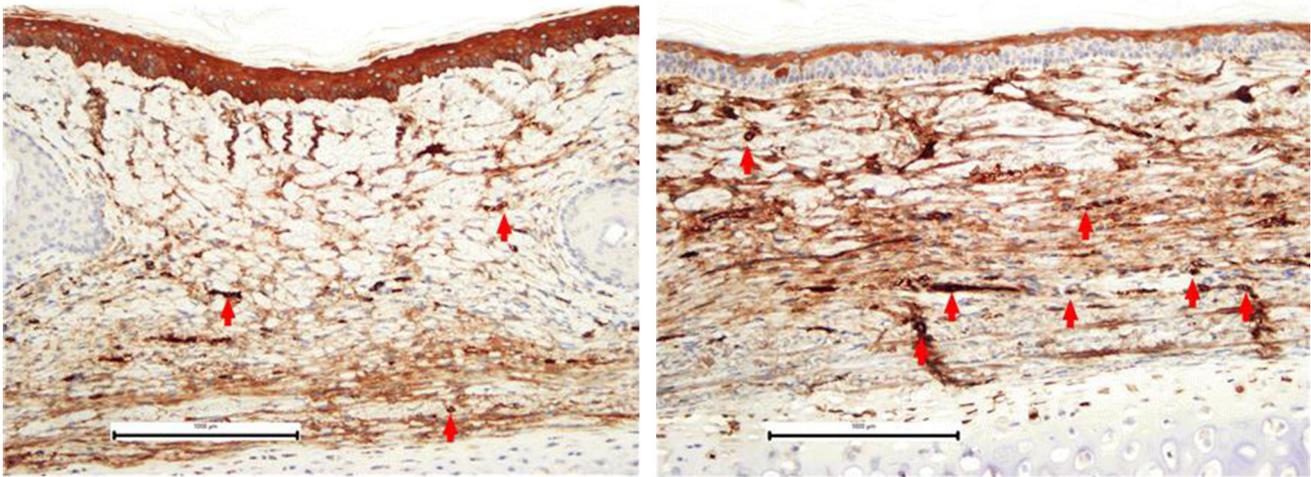


Fig. 5 Immunohistochemical analysis. The specimens were taken 12 days after surgery and subjected to anti-CD31 antibodies, which revealed clear differences in capillary density between the groups (left, control group; right, PDRN group). Capillaries are marked with red arrows

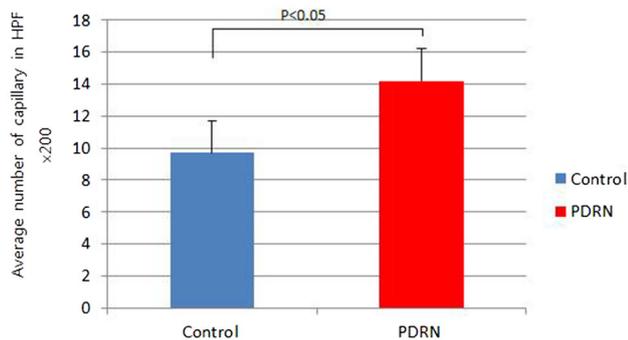


Fig. 6 Comparison of vascular density. Vascular density is defined as the mean number of CD31 + capillaries in a high-power field ($\times 200$). The number of capillaries stained with anti-CD31 antibody was significantly higher in the PDRN group than in the control group (control group, 9.7 ± 2.00 ; PDRN group, 14.2 ± 1.99 ; $p < 0.05$)

It is well known that PDRN acts on purinergic receptors to stimulate tissue regeneration and wound healing [17]. Among the purinergic receptors, PDRN activates adenosine A_{2A} receptors (a P1 subclass) [18]. Looking at this mechanism in more detail, activation of the A_{2A} receptor induces VEGF upregulation, which induces angiogenesis of the surgical site and promotes revascularization. Then, PDRN causes an anti-inflammatory effect through A_{2A} receptor activation. In particular, it has been shown that pro-inflammatory cytokines such as IL-6, TNF- α , and HMGB-1 are reduced, thereby reducing the inflammatory phase which belongs to the second stage of the wound healing process. In previous studies, it was confirmed that it has an effect on not only soft tissue including skin [19] but also treating arthritis [20] and periodontitis [21]. A recent study had confirmed that JNK signaling is involved through cell research, and various molecular sizes of DNA have been shown to be effective in wound regeneration in

which the quality of wound healing is the highest in the PDRN-treated group [22]. Activation of the receptor also acts as a stimulant on cell lines such as osteoblasts, fibroblasts, preadipocytes, and collagen [11, 23]. In another study, PDRN increased tissue oxygenation during local injection to a diabetic foot ulcer [24], promoted angiogenesis, and accelerated recovery of the injection site at the pressure ulcer [25] and the wound healing of graft donor sites [26]. In animal studies, PDRN was shown to induce recovery in artificially created defects or incision lines [19, 27]. Another research reported that injection of PDRN improved viability of random pattern skin flaps on rats [28, 29]. It has also been shown to improve blood flow in peripheral artery occlusive disease in rats [30] and in cases of thermal injury [31]. However, there is no animal study that applied PDRN directly to a composite tissue graft model with the blood supply blocked. Additionally, laser speckle contrast imaging as a method of monitoring blood flow in grafts had not been previously attempted.

In our study, PDRN was found to improve composite graft survival successfully. On postoperative day 12, we demonstrated statistically that the necrotic areas of the grafted tissue in the PDRN group were significantly smaller than those in the control group. This effect of PDRN was also well correlated with our histological and immunohistochemical results; the PDRN group clearly showed a higher capillary density using anti-CD31 antibodies than the control group, indicating more neovascularization in the PDRN group.

Laser speckle contrast imaging is a technology based on time-integrated speckle patterns generated by low-power laser radiation, imaged with a CCD camera, and was used to monitor blood flow in our study because of its feature that enables noninvasive evaluation of blood flow [14].

Laser speckle contrast imaging showed a significant difference in blood flow between the two groups, with higher blood flow in the PDRN group. Neovascularization from the margin is thought to be important for composite graft survival [3]. Consistently, laser speckle contrast imaging showed that the higher signal, which indicates areas with increased blood flow, initially appeared at the periphery of the composite graft and progressively spread by day 12 post-surgery in PDRN-injected animals. Through this, we could confirm that the circulation recovered from the margin despite leaving the skin intact on the back of the ear.

Some limitations of this study were that although 40 grafts from 20 rabbits were studied, the sample size is not large enough and a larger-scale study is required. Also, we could see that PDRN improves survival in chondrocutaneous composite grafts, but because of its characteristics, it is difficult to apply the prospective study to human beings limiting actual clinical application. Therefore, applying PDRN into people who have been treated with composite grafts is likely to require further study with detailed informed consent from the patient. Fortunately, with the exception to hypersensitivity reactions such as nausea or mild rash by local injection, no immunological adverse effects have been identified in clinical/preclinical studies that have been studied so far [17, 32].

With consideration that composite grafts are a favorably used reconstruction choice for facial defects, our study is valuable because it demonstrates our hypothesis that PDRN significantly improves the viability of such graft tissues, providing a potential new approach to using composite grafts. In reconstruction of the face such as the nose, graft failure of the composite graft increases the cosmetic disadvantages and the patient's psychological distress. Therefore, if the survival rate of grafts can be increased by simple local injection alone, it can be said to be of sufficient value.

It appears that PDRN enhances restoration of perfusion by increasing neovascularization, around and at the base of the graft, and exerts a positive effect on graft survival. These properties were demonstrated by both histological and optical methods, employing laser speckle contrast imaging. In conclusion, our study shows that PDRN restores the perfusion of composite grafts by increasing neovascularization around and at the base of the graft and may exert a positive effect on the survival of composite grafts.

Funding This work was supported (in part) by the Yonsei University miraeseondo project of 2017 (2017-52-0064).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Human and Animal Rights The Animal Care and Experiment Committee of Yonsei Wonju University approved the experimental protocol.

Informed Consent For this type of study, informed consent is not required.

References

- Hong JP (2018) Flap classification and applications. In: Neligan PC (ed) Plastic surgery, 4th edn. Elsevier Saunders, Philadelphia, pp 336–432
- Scherer SS, Pietramaggiore G, Orgill DP (2018) Skin graft. In: Neligan PC (ed) Plastic surgery, 4th edn. Elsevier Saunders, Philadelphia, pp 214–230
- Eberlin KR, Busa K, Bae DS, Waters PM, Labow BI, Taghian AH (2015) Composite grafting for pediatric fingertip injuries. *Hand (N Y)* 10:28–33
- Son D, Kwak M, Yun S, Yeo H, Kim J, Han K (2012) Large auricular chondrocutaneous composite graft for nasal alar and columellar reconstruction. *Arch Plast Surg* 39:323–328
- El-Hennawi DM (2001) Cartilage perichondrium composite graft (CPCG) in pediatric tympanoplasty. *Int J Pediatr Otorhinolaryngol* 59:1–5
- Hartman DF, Goode RL (1987) Pharmacologic enhancement of composite graft survival. *Arch Otolaryngol Head Neck Surg* 113:720–723
- Eo S, Hur G, Cho S, Azari KK (2009) Successful composite graft for fingertip amputations using ice-cooling and lipo-prostaglandin E1. *J Plast Reconstr Aesthet Surg* 62:764–770
- Li EN, Menon NG, Rodriguez ED, Norkunas M, Rosenthal RE, Goldberg NH, Silverman RP (2004) The effect of hyperbaric oxygen therapy on composite graft survival. *Ann Plast Surg* 53:141–145
- Tonello G, Daglio M, Zaccarelli N, Sottofattori E, Mazzei M, Balbi A (1996) Characterization and quantitation of the active polynucleotide fraction (PDRN) from human placenta, a tissue repair stimulating agent. *J Pharm Biomed Anal* 14:1555–1560
- Galeano M, Bitto A, Altavilla D, Minutoli L, Polito F, Calo M, Lo Cascio P, Stagno d'Alcontres F, Squadrito F (2008) Polydeoxyribonucleotide stimulates angiogenesis and wound healing in the genetically diabetic mouse. *Wound Repair Regen* 16:208–217
- Montesinos MC, Desai A, Chen JF, Yee H, Schwarzschild MA, Fink JS, Cronstein BN (2002) Adenosine promotes wound healing and mediates angiogenesis in response to tissue injury via occupancy of A(2A) receptors. *Am J Pathol* 160:2009–2018
- Briers JD, Webster S (1996) Laser speckle contrast analysis (LASCA): a non-scanning, full-field technique for monitoring capillary blood flow. *J Biomed Opt* 1:174–179
- Jeon YR, Kang EH, Yang CE, Yun IS, Lee WJ, Lew DH (2014) The effect of platelet-rich plasma on composite graft survival. *Plast Reconstr Surg* 134:239–246
- Choi B, Kang NM, Nelson JS (2004) Laser speckle imaging for monitoring blood flow dynamics in the in vivo rodent dorsal skin fold model. *Microvasc Res* 68:143–146

15. Lee KS, Lim Y, Choi J, Kim NG, Kim JS (2013) Composite graft including bone tissue: a case report of successful reattachment of multiple fingertip oblique amputation. *J Plast Reconstr Aesthet Surg* 66:e43–46
16. Mc LC (1954) Composite ear grafts and their blood supply. *Br J Plast Surg* 7:274–278
17. Squadrito F, Bitto A, Irrera N, Pizzino G, Pallio G, Minutoli L, Altavilla D (2017) Pharmacological activity and clinical use of PDRN. *Front Pharmacol* 8:224
18. Thellung S, Florio T, Maragliano A, Cattarini G, Schettini G (1999) Polydeoxyribonucleotides enhance the proliferation of human skin fibroblasts: involvement of A2 purinergic receptor subtypes. *Life Sci* 64:1661–1674
19. Lee JH, Han JW, Byun JH, Lee WM, Kim MH, Wu WH (2018) Comparison of wound healing effects between *Oncorhynchus keta*-derived polydeoxyribonucleotide (PDRN) and *Oncorhynchus mykiss*-derived PDRN. *Arch Craniofac Surg* 19:20–34
20. Bitto A, Polito F, Irrera N, D'Ascola A, Avenoso A, Nastasi G, Campo GM, Micali A, Bagnato G, Minutoli L, Marini H, Rinaldi M, Squadrito F, Altavilla D (2011) Polydeoxyribonucleotide reduces cytokine production and the severity of collagen-induced arthritis by stimulation of adenosine A_{2A} receptor. *Arthritis Rheum* 63:3364–3371
21. Bitto A, Oteri G, Pisano M, Polito F, Irrera N, Minutoli L, Squadrito F, Altavilla D (2013) Adenosine receptor stimulation by polynucleotides (PDRN) reduces inflammation in experimental periodontitis. *J Clin Periodontol* 40:26–32
22. Hwang KH, Kim JH, Park EY, Cha SK (2018) An effective range of polydeoxyribonucleotides is critical for wound healing quality. *Mol Med Rep* 18:5166–5172
23. Guizzardi S, Galli C, Govoni P, Boratto R, Cattarini G, Martini D, Belletti S, Scandroglio R (2003) Polydeoxyribonucleotide (PDRN) promotes human osteoblast proliferation: a new proposal for bone tissue repair. *Life Sci* 73:1973–1983
24. Kim S, Kim J, Choi J, Jeong W, Kwon S (2017) Polydeoxyribonucleotide improves peripheral tissue oxygenation and accelerates angiogenesis in diabetic foot ulcers. *Arch Plast Surg* 44:482–489
25. Kim JY, Pak CS, Park JH, Jeong JH, Heo CY (2014) Effects of polydeoxyribonucleotide in the treatment of pressure ulcers. *J Korean Med Sci* 29(Suppl 3):S222–227
26. De Aloe G, Rubegni P, Biagioli M, Taddeucci P, Fimiani M (2004) Skin graft donor site and use of polydeoxyribonucleotide as a treatment for skin regeneration: a randomized, controlled, double-blind, clinical trial. *Wounds* 16:258–263
27. Jeong W, Yang CE, Roh TS, Kim JH, Lee JH, Lee WJ (2017) Scar prevention and enhanced wound healing induced by polydeoxyribonucleotide in a rat incisional wound-healing model. *Int J Mol Sci* 18:1698
28. Chung KI, Kim HK, Kim WS, Bae TH (2013) The effects of polydeoxyribonucleotide on the survival of random pattern skin flaps in rats. *Arch Plast Surg* 40:181–186
29. Polito F, Bitto A, Galeano M, Irrera N, Marini H, Calo M, Squadrito F, Altavilla D (2012) Polydeoxyribonucleotide restores blood flow in an experimental model of ischemic skin flaps. *J Vasc Surg* 55:479–488
30. Bitto A, Polito F, Altavilla D, Minutoli L, Migliorato A, Squadrito F (2008) Polydeoxyribonucleotide (PDRN) restores blood flow in an experimental model of peripheral artery occlusive disease. *J Vasc Surg* 48:1292–1300
31. Bitto A, Galeano M, Squadrito F, Minutoli L, Polito F, Dye JF, Clayton EA, Calo M, Venuti FS, Vaccaro M, Altavilla D (2008) Polydeoxyribonucleotide improves angiogenesis and wound healing in experimental thermal injury. *Crit Care Med* 36:1594–1602
32. Lazzarotto M, Tomasello EM, Caporossi A (2004) Clinical evaluation of corneal epithelialization after photorefractive keratectomy in a patients treated with polydeoxyribonucleotide (PDRN) eye drops: a randomized, double-blind, placebo-controlled trial. *Eur J Ophthalmol* 14:284–289

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.