

Acceleration of Wound Healing and Collagen Deposition in Rat Skin by High Voltage Pulsed Current Stimulation

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<Abstract>

The purpose of this study was to investigate the effect of high voltage pulsed current stimulation (HVPCS) on the healing rate of a dermal wound in a rat. We also determined the mechanism of promoting healing by HVPCS. Twenty male Sprague-Dawley rats were randomly divided into two groups; HVPCS group (n=10) and control group (n=10). The HVPCS rats received electrical stimulation with a current intensity of 50 V at 100 pps for a duration of 30 minutes, while the control group was given the same treatment without electricity for a week. The biopsy specimens were fixed in formalin, embedded in paraffin and stained with Masson's trichrome, hematoxylin and eosin (H&E). The fibroblasts and collagen density were counted using a light microscope and computerized image analysis system and calculated as the density and the percent. A Student *t*-test showed a significantly higher wound healing rate of the HVPCS group than control ($t=-4.161$, $p<0.001$). The fibroblasts in the HVPCS group were higher than in the control group ($t=-4.921$, $p<0.001$). The density of collagen in the HVPCS group was also higher than in the control group ($t=-4.367$, $p<0.001$). These results indicate that the HVPCS accelerated the rate of healing in dermal wound, and increased fibroblasts and collagen density in the regenerative dermis. These findings suggest that the HVPCS may activate fibroblasts by alteration of the electrical environment, and it may increase collagen synthesis in the regenerative dermal wound.

KEY WORDS : wound, fibroblast, collagen, electrical stimulation, rat

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Introduction

Since Assimacopoulos (1968) has reported the treatment of ulcers of the leg using electrical stimulation with direct current, there has been substantial evidence that electrical stimulation can promote tissue healing. As non or delayed heal wounds can present many debilitating, long-term problems physically and psychologically. Some clinicians recommended electrical stimulation such as high voltage pulsed current, direct current, pulsed electromagnetic field to treatment of wounds that do not heal normally (Kloth and Feedar, 1988; Feedar et al, 1991; Griffin et al, 1991; Gentzkow, 1993). In recent years, the interest of electrical stimulation for tissue repair has increased. Because of the success of electrical stimulation in clinical trials, the Agency for Health Care Policy and Research recommends that a course of electrical stimulation treatment be considered for stage II, III and IV pressure ulcers that have not responded to other conservative treatment (Bergstrom et al, 1994).

Several studies of human subjects showed that HVPCS promote the healing rate of ulcers. Kloth and Feedar (1988) have reported that HVPCS accelerates the healing rate of stage IV decubitus ulcers, and Feedar et al (1991) have claimed that HVPCS have a beneficial effect on healing stage II, III, and IV chronic dermal ulcers. Griffin et al (1991) have reported that HVPCS enhanced the healing rate of stage II-IV decubitus ulcers in patients with spinal cord injury.

The use of electrical stimulation to promote tissue healing has been reported by many authors. In especially, many investigators to treat wounds in animals and human subjects have used HVPCS. Brown et al (1988) have reported that HVPCS enhanced dermal wound healing rate in rabbits, and Cruz et al (1989) also have reported that HVPCS promoted healing rate of burn in pig model.

The migration, proliferation and activity of the fibroblasts may regulated by an exogenous electricity. Brown and Loew (1994) have reported that the fibroblast exhibit cathode directed motility when exposed to direct current electric fields. Erickson and Nuccitelli (1984), also have reported that the fibroblasts are migrated towards the cathode of the field by extending lamellipodia in the direction. Bourguignon et al (1989) have showed that the fibroblasts are attracted by the negative pole to proliferate and synthesize collagen. These findings suggest that the electricity can influence fibroblast activity

Many preclinical studies have reported that externally applied electrical stimulation can promote healing of soft tissue cause an increase of fibroblast activity and collagen synthesis. However, the previous reports were based on *in vitro* experiment with cultured cells. In this study, consequently, we used the rat full-thickness incision wound model and HVPCS. One week post-wounding, wound healing rate, fibroblast and collagen density was determined *in situ*. We investigated the effects of HVPCS in wound healing rate, fibroblast density and collagen density *in vivo* model of incision wound in rat to provides evidence that the cellular activities of fibroblast are enhanced by HVPCS.

MATERIALS and METHODS

Animals

Twenty male Sprague-Dawley rats weighing 170-210 g were used. Rats were housed one per cage in a room maintained at $22\pm 0.5^{\circ}\text{C}$ with an alternating 12 hours light-dark cycle. Food and water were allowed ad libitum until they were transported to the laboratory approximately an hour before the experiments. We performed all experiments under normal room light and temperature.

Surgical Procedures

Three days before the operation, the hair were shaved from back and abdominal region. Rats were anesthetized by inhalation of halothane, maintained at a concentration of 2-3%. We placed marks 20 mm apart on the skin vertical to the left of the vertebral column. then make a 10 mm length of full-thickness incision with a sterile scalpel #11. The incision area was clean with a sterile gauze pad, and the rat was carefully observed until it had recovered fully from the anesthesia. The incision was not ligated and covered or bandaged.

High Voltage Pulsed Current Stimulation

The animals were randomly assigned into two groups as follows: HVPCS group (n=10), control group (no HVPCS, n=10). The rats were placed in a clear plastic restrainer and rested comfortably. The incision area and abdominal area of rat skin were covered with saline-soaked gauze pads (2 x 2 cm), and carbon-silicone rubber electrodes (2 x 2 cm) were placed over each moist gauze, then secured by a restrainer. Electric line cords were inserted through the window in the restrainer. Positive and negative electric line cords connected the electrode on the back and abdomen, respectively. The HVPCS rats were received electrical stimulation with a current intensity of 50 V at 100 pps for a duration of 30 minutes using a Pulsed High-Volt Stimulator (Intelect[®] HV2, Chattanooga Group Inc., 4717 Adams Rd., P.O.Box 489, Hixson, TN 37343, USA). The polarity was changed after 15 minutes. The wave form of the high voltage pulse current stimulator consists of monophasic, twin-peak pulses that have a fixed pulse duration of $65\mu\text{s}$. For control group, rats were received a sham treatment without power supplied.

Evaluation of Wound Healing

The rats were anesthetized by halothane inhalation. The incision wound length was measured using a caliper. The wound closure was calculated as a percentage of the reduction from the original incision length.

Tissue Sampling and Histochemical Staining

A 10 x 10 mm of the back skin was excised and fixed in 10% phosphate buffered formalin. The tissue sample included subcutaneous tissue and musculature. Following tissue sampling, the rats were sacrificed by CO_2 inhalation. The fixed tissue sections were

dehydrated by ascending graded alcohol series, cleared with xylene using an automatic tissue processor (Citadel 1000, Shandon, Life Sciences International Ltd., Astmoor, Rumcorn, England, WA7 1PR). The tissue sample was embedded in paraffin and cut into 5 μm thick serial sections using a rotary microtome (Rotary Microtome HM 340E, Microm Laborgeräte GmbH, Robert-Bosch-Strasse 49, D-6909 Walldorf, Germany). For each rat skin tissue sample, serial sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT).

Quantification of Fibroblast and Collagen Density

Quantification of fibroblast and collagen density were performed using a computerized image analysis. Images were recorded with a colour CCD camera (IK-642K Toshiba CCD color camera, Toshiba Co., 1-1-1 Shibatori, Minato-Ku, Tokyo, Japan), attached to a light microscope (Olympus BX 50, Olympus Optical Co., Ltd., 2-43-2, Hatagaya, Shibuya-Ku, Tokyo, Japan). The images were analysed with an image analysis software package (Image-Pro[®] Plus, ver 3.1.1, Media Cybernetics, Inc., 8484 Georgia Avenue, Silver Spring, MD 20910, U.S.A.). The fibroblast density was analysed in H & E stained wound sections under x40 objectives. The total regenerated dermal area and the number of fibroblasts were counted. The density of collagen was analysed in Masson's trichrome stained sections under x40 objectives. At this magnifications, each pixel of the computer image corresponded to 4.128 μm and each field represented an area of 0.018 mm^2 of the tissue. The total regenerated dermal area and the area with collagen were measured and subsequently, the area of collagen was expressed as a percentages of the total regenerated dermal tissue.

Data Analysis

For a comparison of the mean rate of wound healing, fibroblasts, densities of collagen between the HVPCS and the control groups, a Student's *t*-test was used. The statistical interpretation was based on a 0.05 significance test level. SPSS WIN (ver 10.0) software was used for the analyses .

RESULTS

General Finding and Wound Healing

One week post-wounding, the wounds treated with HVPCS were suppler and the surface was smoother than those control. In control group, the mean rate of wound healing was $49.20 \pm 22.99\%$. In HVPCS group had mean wound healing rate of $78.95 \pm 22.21\%$. Student's *t*-test showed a significantly higher the rate of wound healing in the HVPCS group than control group ($t = -4.161$, $p < 0.001$). The wounds treated with HVPCS had significantly increased the wound healing rate (Fig. 1).

Density of Fibroblast

The density of fibroblast in the regenerated dermis on H&E stained wound sections of the HVPCS group and the control group are shown in Fig. 2. Most wound showed a completely infiltrated dermal substitute in the H&E stained wound sections. All wounds in

HVPCS group, completely reepithelialized and granulation tissues were nearly replaced by fibrosis and hair follicles were almost healed. However, 70% of the control rat noted epithelial contact and appeared inflammation. In control group, the mean density of fibroblast was $755.84 \pm 223.84/\text{mm}^2$. In HVPCS group had mean density of fibroblast of $1260.39 \pm 356.80/\text{mm}^2$. Student's *t*-test showed a significantly higher the fibroblast density in the HVPCS group than control group ($t = -4.367$, $p < 0.001$). The wounds treated with HVPCS had significantly increased the fibroblast density.

Density of Collagen

The density of collagen in the regenerated dermis on MT stained wound sections of the HVPCS group and the control group were shown in Fig. 3. In control group, the mean density of collagen was $26.91 \pm 11.53\%$. In HVPCS group had mean density of collagen $43.61 \pm 9.87\%$. Student's *t*-test showed a significantly higher the rate of collagen density in the HVPCS group than control group ($t = -4.921$, $p < 0.001$). The wounds treated with HVPCS had significantly increased the collagen density. The regenerated dermis between the edges of wound treated with HVPCS showed thicker collagen bundles organized with preferential orientation parallel to the epidermis (Fig. 3).

DISCUSSION

In this study we have shown that HVPCS can increase the rate of dermal wound healing in rat. We also have evidence that HVPCS can increase the fibroblast and collagen densities *in situ*. We found that the wound healing rate is significantly correlated to fibroblasts and collagen densities.

Previous investigators reported that high voltage monophasic pulsed electrical stimulation with positive polarity enhanced dermal wound healing rate in rabbits (Brown et al, 1988) and pig model.(Cruz et al, 1989). In addition, Kloth and Feedar (1988) reported that HVPCS with 100 pps and 100-175 V accelerates the healing rate of stage IV decubitus ulcers. Feedar et al (1991) claimed that 128 pps pulsed electrical stimulation has a beneficial effect on healing stage II, III, and IV chronic dermal ulcers. Griffin et al (1991) reported that HVPCS with 100 pps and 200 V accelerates the healing rate of stage II-IV decubitus ulcers in patients with spinal cord injury. Our results in accordance with results of their studies on dermal wound in rabbit and pig which showed that HVPCS accelerate wound healing rate.

Normal healing of wound follows the general process of inflammation, proliferation and remodeling. During the initial inflammatory phase of dermal wound repair, fibrin and other components of inflammatory cells and cells derived from the dermis in the subsequent proliferative phase of wound healing. Fibroblasts play a key role in dermal wound repair process and is the major source of collagen production, protein mediators of repair and matrix proteoglycans (Leadbetter, 1992). Recently, several evidences have reported for increase of fibroblast activities by electrical stimulation. Although types of electrical stimulation are difference to high voltage pulsed current, Cheng and Goldman (1998) have reported that in a dermal

wound model, consisting of human skin fibroblasts in collagen matrix, continuous sinusoidal electrical current stimulation elicited a significant increase of [³H]thymidine incorporation over an 8 hour period extending from 16-24 hours after stimulus initiation with 41 mV/m amplitude, 10 Hz. Dunn et (1988) also have reported that fibroblast ingrowth and collagen fiber alignment were increased in collagen sponges stimulated with direct currents between 20 and 100 μ A. Bourguignon and Bourguignon (1987) have reported that the HVPCS increases in synthesis of protein and DNA in fibroblast *in vitro* study. In a previous study, we found that HVPCS with 100 pps, 50 V for 30 minutes enhanced the rate of proliferation and migration of fibroblast from tendon biopsies (Lee et al, 2002). In this study we found that HVPCS with 100 pps, 50 V for 30 minutes increases fibroblast in rat dermal wound model *in situ*.

Fibroblasts proliferate is eventually leading to the synthesis and accumulation of collagen. Collagen functions principally in a structural role, maintaining form and limiting deformation. It also participates in blood clotting, inflammation, and tissue repair (Wang, 1998; Woo et al, 1999). Some investigators have provided evidence that electrical stimulation can increase collagen synthesis in dermis. Thawer and Houghton (2001) have reported that lower doses of electrical stimulation with 5.0 V increased collagen deposition in mice wound. Guler et al (1996) have reported that 1.9 kV/m electric field increased hydroxyproline content in liver tissue of guinea pig, and Canseven and Atalay (1996) also reported that the hydroxyproline content was increased in skin wound tissue by direct microcurrent stimulation which indicated that collagen synthesis is increased by electric current. In our study HVPCS increased collagen deposition in the regenerative dermis in dermal wound of the rat.

The molecular mechanism for the proliferation and migration of fibroblasts by electrical stimulation is not known precisely. The ionic currents act an ionophoretic force on charged proteins and lipids in the cell membrane which exert to redistribution of membrane components. The electrical stimulation can alters the electrical properties of cell membranes. Therefore, the electrical stimulation induces cellular responses including lateral redistribution of membrane proteins such as ion channels and receptors (Brown and Loew, 1994), and changes in intracellular calcium ion concentration. Intracellular Ca⁺⁺ regulates many biological processes including signal transduction cascades, cytoskeletal reorganization, cell orientation and migration, and cell differentiation and proliferation (Bourguignon and Bourguignon, 1987; Cho et al, 1994; Kim et al, 1998). It could be speculated that HVPCS which are similar to the current of injury may accelerate the cellular metabolism by alteration of the electrical micro-environment of fibroblast, and it may increase the proliferation and migration of fibroblast and collagen biosynthesis in regenerative dermis in dermal wound of the rat.

Further investigations with various parameters of HVPCS should be performed to substantiate the optimal parameters and other cells, cytokines and matrix functions to be related to wound healing process.

CONCLUSION

The results of this study revealed a statistically significant higher rate of dermal wound healing, density of fibroblasts and collagen in the regenerative dermis *in situ* in HVPCS rats compared to that in control rats. This result suggests that the HVPCS with 100 pps, 50 V for 30 minutes promoted dermal wound healing in rats. In view of the results, HVPCS may activate fibroblast by alteration of the electrical micro-environment, and it may increase collagen synthesis in the regenerative dermal wound. Further investigations with many groups of animals and various parameters of HVPCS should be performed to substantiate the optimal parameters and other cells, cytokines and matrix functions to be related to wound healing process.

References

1. Assimacopoulos D. Low intensity negative electric current in the treatment of ulcers of the leg due to chronic venous insufficiency. Preliminary report of three cases. *Am. J Surg*, 115(5), 683-687, 1968.
2. Bergstrom N, Allman RM, Alvarez OM, Bennett MA, Carlson CE, Frantz RA, Garber SL, Jackson BS, Kaminski MV, Kemp MG, Krouskop TA, Lewis VL Jr, Maklebust J, Margolis DJ, Marvel EM, Reger SI, Rodeheaver GT, Salcido R, Xakellis GC and Yarkony GM. Treatment of Pressure Ulcers. Clinical Practice Guideline No. 15. U.S. Department of Health and Human Services. Public Health Service, Agency for Health Care Policy and Research, AHCPR Publication No. 95-0652, Rockville, Md, December, 1994.
3. Bourguignon GJ and Bourguignon LYW. Electric stimulation of protein and DNA synthesis in human fibroblasts. *FASEB J*, 1(5), 398-402, 1987.
4. Bourguignon GJ, Jy W and Bourguignon LYW. Electric stimulation of human fibroblasts causes an increase in Ca^{2+} influx and the exposure of additional insulin receptors. *J. Cell Physiol*, 140(2), 379-385, 1989.
5. Brown M, McDonnell MK and Menton DN. Electrical stimulation effects on cutaneous wound healing in rabbits. A follow-up study. *Phys Ther*, 68(6), 955-960, 1988.
6. Brown MJ and Loew LM. Electric field-directed fibroblast locomotion involves cell surface molecular reorganization and is calcium independent. *J Cell Biol*, 127(1), 117-128, 1994.
7. Canseven AG and Atalay NS. Is it possible to trigger collagen synthesis by

- electric current in skin wounds? *Indian J Biochem. Biophys*, 33(3), 223-227, 1996.
8. Cheng K and Goldman RJ. Electric fields and proliferation in a dermal wound model: cell cycle kinetics. *Bioelectromagnetics* 19(2), 68-74, 1998.
 9. Cho MR, Thatte HS, Lee RC and Golan DE : Induced redistribution of cell surface receptors by alternating current electric fields. *FASEB J*, 8(10), 771-776, 1994.
 10. Cruz NI, Bayron FE and Suarez AJ. Accelerated healing of full-thickness burns by the use of high-voltage pulsed galvanic stimulation in the pig. *Ann Plast Surg*, 23(1), 49-55, 1989.
 11. Dunn MG, Doillon CJ, Berg RA, Olson RM and Silver FH. Wound healing using a collagen matrix: effect of DC electrical stimulation. *J Biomed Mater Res*, 22(A2 Suppl), 191-206, 1988.
 12. Erickson CA and Nuccitelli R. Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. *J Cell Biol*, 98(1), 296-307, 1984.
 13. Feedar JA, Kloth LC and Gentzkow GD. Chronic dermal ulcer healing enhanced with monophasic pulsed electrical stimulation. *Phys Ther*, 71(9), 639-649, 1991.
 14. Gentzkow GD. Electrical stimulation to heal dermal wounds. *J Dermatol Surg Oncol*, 19(8), 753-758, 1993.
 15. Griffin JW, Tooms RE, Mendius RA, Clifft JK, Vander Zwaag R and el-Zeky F. Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. *Phys Ther*, 71(6), 433-442, 1991.
 16. Guler G, Atalay NS, Ozogul C and Erdogan D. Biochemical and structural approach to collagen synthesis under electric fields. *Gen Physiol Biophys*, 15(6), 429-440, 1996.
 17. Kim YV, Conover DL, Lotz WG and Cleary SF. Electric field-induced changes in agonist-stimulated calcium fluxes of human HL-60 leukemia cells. *Bioelectromagnetics*, 19, 366-376, 1998.
 18. Kloth LC and Feedar JA. Acceleration of wound healing with high voltage, monophasic, pulsed current. *Phys Ther*, 68(4), 503-508, 1988.
 19. Leadbetter WB. Cell-matrix response in tendon injury. *Clin Sports Med*, 11, 533-578, 1992.

20. Lee JH, Jekal Seung-Joo and Park Rae-Joon. Enhance of Migration and Proliferation of Cells from Tendon Biopsies by High Voltage Pulsed Current Stimulation. J Korean Soc Phys Ther, 14(2), 53-58, 2002.
21. Thawer HA and Houghton PE : Effects of electrical stimulation on the histological properties of wounds in diabetic mice. Wound Repair Regen, 9(2), 107-115, 2001.
22. Wang ED : Tendon repair. J Hand Ther, 11(2), 105-110, 1998.
23. Woo SL, Hildebrand K, Watanabe N, Fenwick JA, Papageorgiou CD and Wang JH. Tissue engineering of ligament and tendon healing. Clin Orthop, 367, S312-323, 1999.

<국문초록>

고전압맥동전류자극이 흰쥐 피부 창상치유와 교원질 축적에 미치는 효과

원광보건대학 물리치료과

이 재형

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고전압맥동전류자극이 흰쥐 피부의 창상치유와 섬유모세포 및 교원질 밀도에 미치는 영향을 규명할 목적으로 본 실험을 시행하였다. Sprague-Dawley계 수컷 흰쥐 20마리를 10마리씩 무작위로 고전압맥동전류자극군과 대조군에 배치하고 등에 10 mm의 전층 절개상을 만들었다. 고전압맥동전류자극군은 창상 위에 전극을 부착한 후 맥동빈도 100 pps, 맥동기간 65 μ s, 강도 50 V로 1일 30분씩 1주 동안 자극하였으며 대조군은 전극만 부착하고 전류를 통전시키지 않았다. 1주 후 창상 길이를 측정하고 창상부위의 피부를 적출하여 포르말린에 고정하고 파라핀 포매 절편을 제작하여 Massons trichrome 및 hematoxylin and eosin (H&E) 염색을 하였다. 이 표본을 고배율 현미경하에서 관찰한 다음 현미경에 부착된 CCD 카메라를 통해 개인용 컴퓨터로 보내 영상분석 프로그램을 이용하여 섬유모세포와 교원질 밀도를 측정하고 비율을 산출하였다. 이를 구간 t -검정한 결과 고전압맥동전류자극군의 창상치유율, 섬유모세포 및 교원질 밀도가 대조군보다 통계학적으로 유의하게 높게 나타났다 ($p < 0.001$). 이러한 결과는 고전압맥동전류자극이 흰쥐의 피부 창상 치유를 촉진시켰음을 보여 주었고 이는 전기자극에 의해 섬유모세포가 활성화되어 교원질 합성이 증가함에 따른 것으로 사료된다.

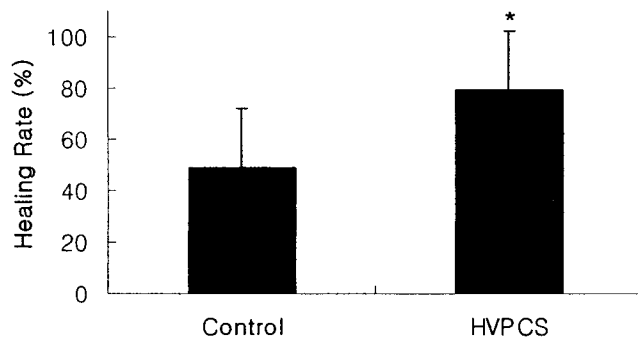


Fig 1. Comparison of the wound healing rate between the control and the HVPCS treated rat at 7 days after wounded. The wound healing rate of the HVPCS was significantly higher than the control ($p < 0.001$). The bar represents the standard deviation of values from 10 rats.

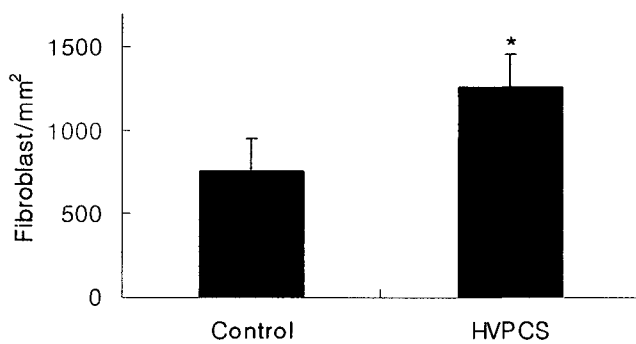
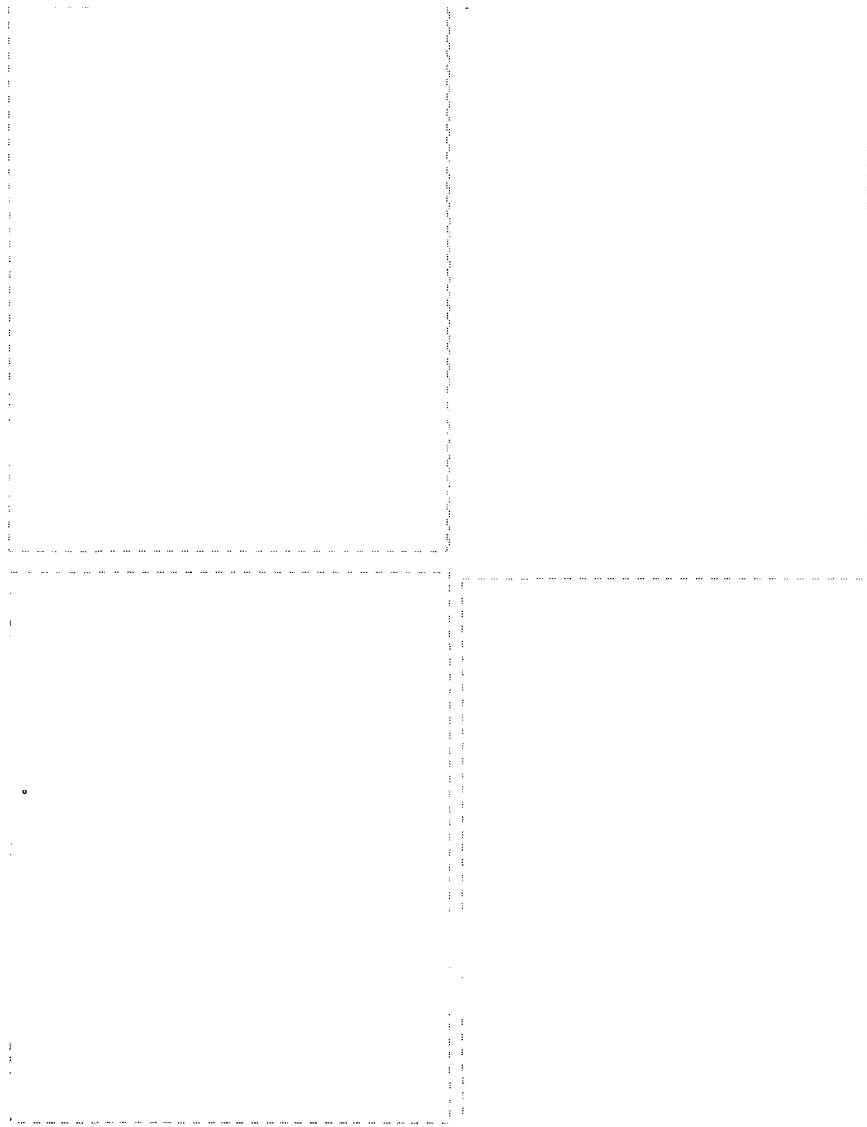


Fig. 2. The fibroblast (arrow) can be seen in the regenerated dermis of wound in the control (A) and the HVPCS (B) at 7 days after incision. A thick band at the top is epidermis (ED) in the dermis and granulation tissue (G). Hematoxylin and eosin stained x100 (A1 and B1) and x400 (A2 and B2). The wounds treated with HVPCS had significantly higher fibroblast density in the regenerated dermis than in the control wound ($p < 0.001$).

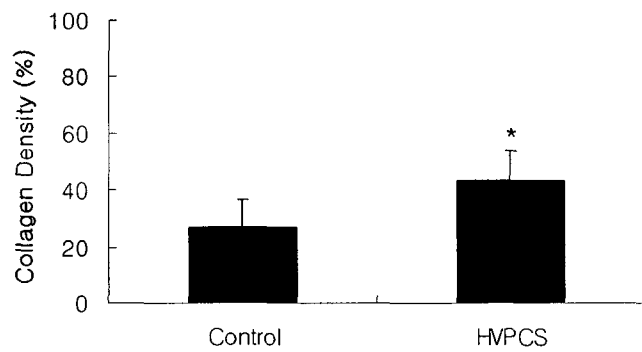


Fig. 3. The bluish-stained collagen (arrow) can be seen in the regenerated dermis of incised wound in the control (C) and the HVPCS (D) at 7 days after incision. Masson-trichrome stain. x100 (C1 and D1) and x400 (C2 and D2). The wounds treated with HVPCS had significantly higher collagen density in the regenerated dermis than in the control wound ($p < 0.001$).