

The 33rd Annual Meeting of the Japanese Society for Wound Healing

Place: Keyaki (University Hall), West Chiba Campus, Chiba University, Chiba, Japan

Date: December 9-10, 2003

Congress President: Takenori Ochiai, Professor at Department of Academic Surgery,
Chiba University Graduate School of Medicine

General I

Moderated by Yoshihide Otani

01 A case of lower leg osteomyelitis successfully treated with Trafermin spray
Takashi Endo, Ibaraki

02 Chondrogenic and osteogenic differentiation of adipose tissue derived stem cells isolated from GFP transgenic mice
Rei Ogawa, Tokyo

03 Expression of the FGF family and Egr-1 in a wound-healing model (Reorganized small intestine from fetal mouse as an in vitro wound healing model)

Hironobu Yonekawa, Saitama

04 Disturbance of wound healing affects the defensive mechanism at the base of the gastric mucosa

Masashi Yoshida, Tokyo

05 Gene expression of liver regeneration after partial hepatectomy in mice lacking type tumor necrosis factor receptor

Tetsuya Shimizu, Kanagawa

General II

Moderated by Yoshihiro Takami

06 Down-regulation of TFF expression by TNF- α in gastric epithelial cells
Yoichiro Fujii, Tochigi

07 Mechanism of impairment in wound healing of electron beam-irradiated rat skin

Kenji Sugiyama

08 Preventive effect of fibroblast growth factor and hepatocyte growth factor on ceramide induced dysfunction human small intestinal cells

Ryo Yamaguchi, Tokyo

10 The expression of human HtrA1 in keloid tissue

Mika Ikeda, Kyoto

General III

Moderated by Takehisa Iwai

11 Muscular flap treatment for intractable ulcer with artificial vessel exposure after a surgery for thoracic aortic aneurysm

Toshihiko Yamauchi, Fukuoka

12 Wound healing and scar formation on the skin of the chest wall and the lower leg after cardiac bypass surgery

Hajime Takahashi, Tokyo

13 Involvement of KGF in the wound healing process of tracheal cartilage in rat longitudinal tracheotomy model

Takafumi Abo, Nagasaki

14 Problems of lower extremity amputation levels

Satoru Tamai, Tokyo

15 Fetal dermal mesenchymal cells possess an ability to regenerate dermis in the reconstitution model of scid mouse

Kazuo Kishi, Tokyo

General IV

Moderated by Yoko Yoshimura

16 A retrospective study of vacuum assisted closure in Japan

Suguru Miyamura, Hyogo

17 Chemical analysis of the surface of silicone modified by oxygen plasma treatment for cell adhesion

Tai Yamada, Aichi

18 Effect of oxygen plasma treatment for cell adhesion properties on silicone surface

Ikuko Ohsugi, Aichi

19 Comparison of bacteria-retaining ability of wound dressings

Masahiro Tachi, Tokyo

General V

Moderated by Yoshihiko Maehara

20 Reduction of ischemia-reperfusion injury to skin flaps by monoclonal antibody to leukocyte endothelial adhesion molecule-1

Yasuyoshi Tosa, Tokyo

21 Chitosan sponge with photocrosslinkable chitosan hydrogel stimulates large and impaired wound healing in rats

Toshiaki Ishizuka, Saitama

22 Liver regeneration after resection of multiple liver cysts

Taketoshi Suehiro

General VI

Moderated by Nobutoshi Andou

26 Lung vitamin E depletion resulting from burn and smoke inhalation in sheep

Katsumi Shimoda, Tokyo

27 Comparative study of non-invasive vascular imaging techniques in the evaluation of chronic venous leg ulcers

Takashi Yamaki, Tokyo

General VII

Moderated by Chifumi Yoshida

30 A case of ulcerative colitis with peristomal pyoderma gangrenosum

Tomoe Ebata, Chiba

31 Important factors in the healing of a pressure ulcer in the ischium of a spinal-cord-injury patient whose pressure ulcers recurred over 26 years

Ayumi Sugai, Osaka

Symposium I-1: Pathophysiology and Treatment of Pressure Ucer

Moderated by Koichi Hirata

S1-01 The study of clinical usefulness of basic FGF

Masahiro Hamanoue, Kagoshima

S1-02 LPA and PDGF stimulated contraction of collagen matrices involves neither by myosin light chain phosphorylation nor by MLC kinase

Masatoshi Abe, Gunma

S1-03 Recombinant G-CSF induces downregulation of IL-12 transcription after partial hepatectomy

Jiro Okiyama, Hiroshima

S1-04 Non-invasive approach for refractory enterovaginal fistula

Hidefumi Nishimori, Osaka

S1-05 Attempt to improve wound healing considering the timing of suture removal and the dressing

Toshihiro Kitte, Oita

Symposium I-2: Pathophysiology and Treatment of Pressure Ucer

Moderated by Toshimasa Asahara

S1-06 Sterilized acellular allogeneic dermal matrix for scaffold of cultured composite skin

Jingping Guo, Tokyo

S1-07 Regulation of VEGF-induced angiogenesis by MMPs

Takayuki Shiomi, Tokyo

S1-08 Skin Regeneration by human mesenchymal stem cells and basic fibroblast growth factor

Hiroshi Nakagawa, Nagasaki

S1-09 Dispersion of pressure to the head in brain/neuro-surgery

Chika Anzai

Symposium I-3: Pathophysiology and Treatment of Pressure Ucer

Moderated by Hiroyuki Kuwano

S1-10 Administration of basic fibroblast growth factor (bFGF) for intractable rectovesical fistula – a case report

Ryoichi Onozato, Gunma

S1-11 Basic fibroblast growth factor accelerates apoptosis in acute incisional wound healing and reduces scar formation

Yoshikiyo Akasaka, Tokyo

S1-12 Interaction between human mesenchymal stem cells and basic fibroblast growth factor or other cell types

Kozo Akino, Nagasaki

S1-13 Open wound integrated regenerative therapy with topical bFGF (Trafermin) for deep soft tissue defects with bone exposure

Norihiko Ohura, Saitama

S1-14 Modification of wound healing process using basic fibroblast growth factor

Ichiro Ono, Hokkaido

Symposium I-4: Pathophysiology and Treatment of Pressure Ucer

Moderated by Takashi Tajiri

S1-15 Wound bed regeneration by autologous unfractionated bone marrow transplantation

Sachio Kouraba, Fukui

S1-16 Accerelation of wound healing with PDWHF and cultured cells

Kazutaka Soejima, Tokyo

S1-17 Viability and function of autologous and allogeneic fibroblasts seeded in dermal substitutes after implantation

Naoki Morimoto, Kyoto

S1-18 Comparison between omental implantation and omental patch for perforated acetic acid-induced gastric ulcer in rats

Yasunori Matoba, Kanagawa

Symposium II: Basic Research and Clinical Approach for Impaired Wound Healing

Moderated by Takashi Nakazuka

S2-01 Negative-pressure dressings in the treatment of infected pressure ulcers

Tsukasa Isago, Tokyo

S2-02 Analysis of pathophysiology of pressure ulcer in a microcirculatory model

Shigeru Ichioka, Saitama

S2-03 How to treat acute phase pressure ulcers with necrotic tissue

Kunio Tsukada, Toyama

S2-04 Oxygen hyperbaric pressure therapy (OHPT) for decubitus ulcers

Takao Fukai, Saitama

**GENERAL SESSION I
MODERATED BY YASHIHIRO OTANI
001****A CASE OF LOWER LEG OSTEOMYELITIS SUCCESSFULLY TREATED WITH TRAFERMIN SPRAY**

Takashi ENDO MD, PhD

Plastic Surgery, Institute of Clinical Medicine, University of Tsukuba

Aim: Chronic osteomyelitis with pus discharge is very difficult to treat. This time we experienced the case of lower leg osteomyelitis, which we have treated with Trafermin spray. The result was excellent and beyond our expectation, so we will report this case.

Case: 63 years, male. He sustained the open fracture injury of his right foot by a fallen rocks and resulted in the osteomyelitis. Then he was introduced to our hospital. Operation was done under spinal anesthesia, the infected bone and covering skin were excised fully and the defect (4×3×3 cm) was left open. After 2 weeks of dressing change, we began to use the Trafermin spray.

Result: At the first 3 weeks, evident change was not recognized, but good granulation formation started 4 weeks later. This tendency has continued for a while and the defect completely closed 5 months later.

Discussion: We have experienced the case of lower leg osteomyelitis, which showed the dramatic improvement. Through this case, we have reconfirmed that rich blood supply around the defect is essential for the healing process when we use the Trafermin spray.

002**CHONDROGENIC AND OSTEOGENIC DIFFERENTIATION OF ADIPOSE TISSUE-DERIVED STEM CELLS ISOLATED FROM GFP TRANSGENIC MICE**Rei Ogawa¹, Hiroshi Mizuno¹, Atsushi Watanabe², Makoto Migita², Takashi Shimada², Hiko Hyakusoku¹¹Department of Plastic and Reconstructive Surgery, ²Department of Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, Japan

Aim: Taking advantage of homogeneously marked cells from green fluorescent protein (GFP) transgenic mice, we have previously demonstrated that bone marrow derived stromal cells (BSCs) differentiate into a variety of cell lineages both in vitro and in vivo. In the present study, we extend this approach to characterize adipose tissue derived stromal cells (ASCs), sometimes called processed lipoaspirate (PLA) cells.

Methods: ASCs were isolated from inguinal fat pads of GFP mice. After the primary culture in control medium, the cells were incubated in osteogenic and chondrogenic medium for 2 to 4 weeks, respectively. Osteogenic and chondrogenic differentiation were assessed by the special staining and RT-PCR.

Results: ASCs incubated in osteogenic medium were stained positively for von Kossa and alkaline phosphatase staining. RT-PCR analysis showed the Expression of osteocyte related genes. Positive staining cells for alcian blue and the expression of chondrocyte related genes were found in ASCs incubated in chondrogenic medium.

Conclusions: These findings suggest that ASCs derived from GFP transgenic mice have both osteochondrogenic potential in vitro. Since this cell population can be easily identified through fluorescence microscope, it may be an ideal source of ASCs for further experiments of stem cell biology and tissue engineering.

003**EXPRESSION OF THE FGF FAMILY AND EGR-1 IN A WOUND HEALING MODEL REORGANIZED SMALL INTESTINE FROM FETAL MOUSE AS AN IN VITRO WOUND HEALING MODEL**Hironobu Yonekawa,¹ Eiko Murata,² Keiko Fujita,² Akira Satomi,¹ and Masumi Akita³¹Department of Pediatric Surgery, ²Department of Anatomy, and ³Division of Morphology, Saitama Medical School

Aim: In the present study, expression of members of the fibroblast growth factor (FGF) family and early growth response gene 1 (Egr-1), known as a transcription factor, was analyzed by reverse transcription polymerase chain reaction (RT-PCR).

Methods: After the mouse fetal small intestine was divided into single cells with collagenase-disperse, the cells were cultured. After reorganized small intestine were resected with a knife, expression of mRNAs from FGF family and Egr-1 was analyzed by RT-PCR.

Results: The wound surface was covered with epithelial cells by 24 hours after resection. Among members of the FGF family, mRNAs from FGF-1, -2, -5, and -7 were expressed. Egr-1 was expressed as early as 15 minutes after resection. Egr-1 is thought to be a transcription factor that induces the expression of FGF-2, other than factors involving wound healing, such as PDGF and TNF- α .

Conclusions: In the present study, it was determined that this structure is a potential model for studying the roles of transcription factor and growth factors expressed in association with damage and wound healing.

References:

1. Hironobu Yonekawa, Eiko Murata, Masumi Akita, Akira Satomi Reorganized small intestine from fetal mouse as an in vitro wound-healing model. *Journal of Gastroenterology* 2003;38(5):442-450.

004**DISTURBANCE OF WOUND HEALING AFFECTS THE DEFENSIVE MECHANISM AT THE BASE OF THE GASTRIC MUCOSA**Masashi Yoshida¹, Go Wakabayashi¹, Hideki Ishikawa¹, Motohide Shimazu¹, Minoru Tanabe¹, Koichiro Kumai^{1, 2}, Tetsuro Kubota¹, Yoshihide Otani¹, Yoshiro Saikawa¹, Kaori Kameyama³, Masaki Kitajima¹¹Department of Surgery, Keio University School of Medicine, Tokyo, Japan²Center for Diagnostic and Therapeutic Endoscopy, Keio University School of Medicine, Tokyo, Japan³Department of Pathology, Keio University School of Medicine, Tokyo, Japan

In vivo microscopy was performed to observe the basal region after thermal injury to the back skin of rats. A donor of nitric oxide, 3-morpholinosydnonimine hydrochloride (SIN-1), or a serine protease inhibitor, camostat mesilate, was administered. Antivascular endothelial growth factor (VEGF) neutralizing antibody was administered 5 hours after thermal injury (anti-VEGF group). Postcapillary venules could be observed in the basal region of the gastric mucosa (PV-BGM). The PV-BGM was dilated 5 hours after thermal injury, and it was reduced by the administration of SIN-1 or pretreatment with camostat mesilate. In the control group, the erosions did not reach the basal region of the gastric mucosa. Most of the erosions healed within 72 hours. Delayed healing was observed in the anti-VEGF group. In the anti-VEGF group, exudation and congestion in the basal region was observed at 24 hours, and ulcer formation was observed at 72 hours after thermal injury. The blood flow of the PV-BGM is thus speculated to increase when the mucosal superficial circulation is disturbed. The PV-BGM can contribute to the defensive mechanisms in the basal region of gastric mucosa. The abnormal process of the healing may disturb the defensive mechanism at the base of the gastric mucosa, thereby result in ulcer formation.

005

GENE EXPRESSION OF LIVER REGENERATION AFTER PARTIAL HEPATECTOMY IN MICE LACKING TYPE TUMOR NECROSIS FACTOR RECEPTOR

Tetsuya Shimizu¹, Shinji Togo¹, Hirochika Makino¹, Kuniya Tanaka¹, Yoji Nagasima², and Hiroshi Shimada¹

Department of Gastroenterological Surgery¹ and Department of Molecular Pathology and Oncology², Yokohama City University Graduate School of Medicine, Yokohama, Japan

Aim: To investigate whether TNF- is necessary for hepatocyte proliferation, we study liver regeneration after partial hepatectomy in mice lacking TNF receptor-1.

Methods: TNF receptor type-1 knockout mice and wild-type mice were subjected to two-thirds partial hepatectomy (PHx). Liver regeneration was evaluated by assessing liver weights and Ki67 immunohistochemistry. Riken cDNA microarray analysis was performed on liver samples from mice undergoing PHx to compare clearly differentiated mouse PHx models (TNFR-1 knockout mice-K group, and wild type mice-W group).

Results: The cumulative survival after PHx in K group was lower than in W group. The mortality rate in K group during the first 3 days after PHx was higher (33%) than in W group. The time to regain the liver weight in K group was 14 days and 7 days in W group. The plasma IL-6 levels in K type at 3 hr was significantly higher than in W group. The Ki67 expression in K group at 4 days was lower than in W group. LPS, Toll like receptor 4 precursor and MAPK 8 interacting protein in K group was higher than in W group. For cell cycle-regulated genes, cyclin D1, NFB light chain and TNF receptor super family membrane 1a in K group was lower than in W group.

Conclusions: Lack of TNF- signaling through TNF receptor type 1 suppresses liver regeneration after partial hepatectomy in spite of enhancement of LPS-JNK pathway, no TNF- and IL-6 pathway.

**GENERAL SESSION II
MODERATED BY YASHIHIRO TAKAMI**

006

DOWN-REGULATION OF TFF EXPRESSION BY TNF- α IN GASTRIC EPITHELIAL CELLS

Y Fujii, T Shimada, A Koitabashi, T Hashimoto, K Hosaka, K Tabei, T Namatame, M Yoneda, H Hiraishi, and A Terano

Department of Gastroenterology and Institute for Medical Science, Dokkyo University School of Medicine, Mibu, Tochigi 321-0293, Japan

Aim: Trefoil factor family (TFF) peptides are known to facilitate wound healing in gastric mucosa. However, the regulatory mechanisms of gastric TFF expression are not fully understood yet. In this study, we examined the effect of TNF- α on TFF1 and TFF2 expression in gastric epithelial cells.

Methods: MKN45 cells were used. TFF mRNA expression was analyzed by real-time quantitative RT-PCR. Promoter sequences of TFF1 gene (-956 to +36) and TFF2 gene (-912 to +24) were inserted into pGL3 vector and reporter gene assays were performed. NF- κ B activity was monitored by using a NF- κ B responsive element-driven reporter vector.

Results: (1) TNF- α (0.1-30 ng/ml) down-regulated TFF1 and TFF2 mRNA expression in a dose-dependent manner. (2) Reporter gene assays also confirmed the down-regulation of TFF1 and TFF2 gene transcription by TNF- α . (3) TNF- α activated NF- κ B. (4) Overexpression of dominant negative I κ Bz prevented both TNF- α -induced NF- κ B activation and TNF- α -induced down-regulation of TFF expression.

Conclusions: TNF- α down-regulates gastric TFF expression through NF- κ B pathway, suggesting that TFF expression is sensitive to inflammatory stimuli.

007

MECHANISM OF IMPAIRMENT IN WOUND HEALING OF ELECTRON BEAM-IRRADIATED RAT SKIN

Kenji Sugiyama¹, Eri Nakatsuji¹, Atsushi Ochiai², Hiroyasu Esumi³, and Yasuhiro Matsumura¹

¹Investigative Treatment Division, and ²Pathology Division, ³National Cancer Center Research Institute East, Kashiwa, Chiba, Japan

Aim: Preoperative radiotherapy for head and neck or colon cancer frequently prevents the following surgical operation, since effective dose of ionized radiation induces wound complication. To make the operation possible, causes for the impaired wound healing were clarified.

Methods: Thighs of F344 rats were irradiated with electron beam (30 Gy). Seven days after the irradiation, full-thickness skin of the thigh was cut by punch biopsy (4-mm diameter). Healing process was determined by measuring size of wound opening. Cytokeratin 14, smooth muscle actin (a marker of myofibroblasts), and integrin beta1 were detected by immunohistochemical analyses.

Results: The wounds in the nonirradiated rats healed almost completely on day 7, whereas repair of epidermis was impaired in the irradiated skin. Numbers of hair follicles and microvessels decreased, and myofibroblasts were almost missing in the scar and dermis adjacent to the wounds of irradiated skins. Proliferation indexes of epidermal basal cells were much higher in irradiated skins than those in nonirradiated skins, however, these basal cells did not express cytokeratin 14, concomitant with the absence of integrin beta1 in basement membrane of the irradiated epidermis. On the other hand, apoptosis appeared in the dermal follicular cells of hair bulbs, 18 h after the irradiation. On day 3, structures of hair follicles were destructed.

Conclusions: Reepithelialization of wounds was blocked in the irradiated skin. Since epidermal basal cells are supplied from stem cells in the hair bulge during cutaneous wound healing, one of causes for the impaired wound healing may be insufficiency of epidermal stem cells.

008

PREVENTIVE EFFECT OF FIBROBLAST GROWTH FACTOR AND HEPATOCYTE GROWTH FACTOR ON CERAMIDE-INDUCED DYSFUNCTION HUMAN SMALL INTESTINAL CELLS

Ryo Yamaguchi^{1,2}, Yoshihiro Takami², Takuya Iida², and Syuij Shimazaki³

BCS inc¹, Plastic Surgery², and Critical Care Medicine³, Kyorin University School of Medicine

Purpose: Gastrointestinal (GI) damages associated with multiple organ failure is a fatal complication. The injured function of GI tract causes malnutrition and the bacterial translocation. So the functional recovery of GI tract is quite important for treating such cases. In the present study, we investigated whether basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) could recover the injured functions of cultured intestinal cells.

Method: Human small intestinal cells were cultured in a 10% FCS added DMEM. The cellular damages were induced by the treatment with various concentrations of cell permeable C6-ceramide (0-20 M). Growth factors (10 ng/ml bFGF or 50 ng/ml HGF) were added to the medium simultaneously or 2 days after the beginning of the ceramide treatment. After 4 days of the treatment, cell growth and apoptosis induction were investigated.

Result and Conclusion: Ceramide inhibited cell growth and induced apoptosis in a dose-dependent manner. Both growth factors significantly improved the cell growth and reduced apoptosis. These effects were also observed when the growth factor treatment was delayed 2 days after the ceramide treatment. It was found that bFGF was more significant than HGF on these protective effects. It is suggested that growth factor treatment may be a useful therapeutic option to improve GI tract dysfunction.

References:

1. Yanai R, et al.: Invest Ophthalmol Vis Sci, 43:2122-2126, 2002
2. Ishihara H, et al.: J Invest Dermatol, 115:1065-1071, 2000

010

THE EXPRESSION OF HUMAN HTRA1 IN KELOID TISSUE

Mika Ikeda, Motoko Naito, and Shigehiko Suzuki

Department of Plastic and Reconstructive Surgery, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho Shogoin Sakyo-ku, Kyoto 606-8507, Japan

Aim: To investigate the expression of human HtrA1 in keloid lesions, and to clarify a possible role of human HtrA1 in keloid pathogenesis.**Methods:** Total RNA was isolated from six keloids and two normal skins by single-step method. The expression level of human HtrA1 was examined by using Northern blot analysis. Keloid and normal skin tissue samples were fixed in paraformaldehyde, and paraffin sections were obtained. Immunohistochemical analysis was performed with anti-human HtrA1 polyclonal antibody.**Results:** The mRNA level of human HtrA1 was markedly elevated in keloid samples, compared with normal skin. Using immunohistochemical analysis, fibroblast-like cells abundantly found in the margin of keloid lesions, were stained with anti-human HtrA1 antibody. No human HtrA1 staining of fibroblasts in normal skin was observed. Interestingly, no significant staining was detected in hypertrophic scar lesions, which is a similar dermal disease to keloid.**Conclusion:** Human HtrA1 expression was found to be up-regulated in keloid lesions, especially in their margin, compared to normal skins and hypertrophic scars. Our data suggest that human HtrA1 could play a critical role in an expression of keloid specific phenotype and in the development of keloid lesions.

012

WOUND HEALING AND SCAR FORMATION ON THE SKIN OF THE CHEST WALL AND THE LOWER LEG AFTER CARDIAC BYPASS SURGERY

Hajime Takahashi, Daisuke Kurata, Masaki Takeuchi¹, Kenji Sasaki¹, and Motohiro Nozaki²

Department of Plastic Surgery, Tokyo Metropolitan Toshima Hospital, 33-1

Sakae-cho, Itabashi-ku, Tokyo 173-0015,

Department of Plastic Surgery, Nihon University¹,Department of Plastic Surgery, Tokyo Women's Medical University²**Introduction:** Many patients receiving heart surgery have extensive operation scars on the skin surface of the chest wall and lower leg. In some cases there are long lasting open wounds in the lower leg after vein harvesting. Some patients complain of pain and itching and we can recognize hypertrophic or extensive scars, which we have to treat.**Case and result:** We treated 16 patients (11 men and 5 women) ranging in age from 47 to 86. They were receiving many drugs such as anticoagulants, thus wound healing was delayed. In these cases, we choose not to resect tissue but apply ointment to remove necrotic tissue, and prepare good granulation tissue for smooth epithelization. Then, we employ prophylactic measures against hypertrophic scar formation such as tape containing steroid, silicon gel sheets and skin tapes as well oral medicine (toranilast) for the same purpose. Because of they have had to continuously take many oral medicines, these patients often do not want to take additional oral medicine. This is one point of difference in the treatment of heart surgery patients and patients with other types of wounds.

GENERAL SESSION III

MODERATED BY TAKEHISA IWAI

011

MUSCULAR FLAP TREATMENT FOR INTRACTABLE ULCER WITH ARTIFICIAL VESSEL EXPOSURE AFTER A SURGERY FOR THORACIC AORTIC ANEURYSM

T Yamauchi, K Kiyokawa, Y Tai, Y Inoue, and Y. Kizuka

Department of Plastic and Reconstructive Surgery and Maxillofacial Surgery, Kurume University School of Medicine, 67 Asahimachi, Kurume, Fukuoka 830-0011, Japan

Aim: To heal an exposed artificial vessel due to mediastinitis after the replacement of thoracic aortic aneurysm.**Methods:** The exposed vessel is washed thoroughly, wrapped in a muscular flap, and a free skin graft is grafted over it to close.**Results:** An 81-year-old male repeatedly had mediastinum after the replacement of aneurysm, and it was not cured with muscular fillings, and a rubor on the chest developed to a subcutaneous hematoma. The lesion was closed with a patch on the artificial vessel, the mediastinal abscess was washed, and the opening was closed by using latissimus dorsi muscular flap and split-thickness skin graft. No recurrence has occurred for 3 years. A 69-year-old male developed mediastinitis after the replacement of thoracic arch aortic aneurysm, and the wound reopened and the vessel exposed. Because conservative treatment did not help, pectoralis major muscular flaps with the perforating branches of the internal thoracic artery and vein as the pedicle were elevated from the both sides, filled the mediastinal space and a split-thickness skin graft was grafted. No recurrence has occurred for one year.**Conclusions:** Artificial vessels that developed infections are usually removed. However, we had good results by washing the lesion and wrapping it with muscular flap. By considering the size of surgical invasion and high death rate associated with the re-plantation of artificial vessel or the opening of omentum, utilization of muscular flap should be considered as the first choice treatment.

013

INVOLVEMENT OF KGF IN THE WOUND HEALING PROCESS OF TRACHEAL CARTILAGE IN RAT LONGITUDINAL TRACHEOTOMY MODEL

Takafumi Abo, Keitarou Matumoto, Takatomo Yamayosi, Takeshi Nagayasu, Sinich Izumi, Yosataka Hisikawa, and Takehiko Kouji

Division of Surgical Oncology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Aim: To address whether KGF (Keratinocyte Growth Factor) and KGFR (keratinocyte Growth Factor Receptor) expression is involved in the wound healing process of tracheal cartilage in rat longitudinal tracheotomy model. And to expose the new strategy to control the process utilizing KGF and its related compounds.**Method:** In this study, we incised rat trachea longitudinally for three cartilage rings, and harvested trachea from rat on 0, 1, 3, 5, 7 days after operation. We made horizontal section and observe them with immunohistochemistry.**Result:** The cartilage defect was filled with chondrocytes originated from the perichondrium. KGF expression was observed in chondrocytes, perichondrium and fibroblasts in injured area. The expression of KGF was upregulated immediately after the operation, reaching a peak at 3-5 days.**Future approach:** Further examination on temporal and spatial pattern of KGF and KGFR expression. Quantitative analysis of the expression of KGF and KGFR in relation to cell kinetics. Attempts to manipulate the kinetics of wound healing using KGF and its related compounds.

014

PROBLEMS OF LOWER EXTREMITY AMPUTATION LEVELS

Satoru Tamai, Yoshinori Inoue, Norihide Sugano, Masayuki Hirokawa, Masatoshi Jibiki, Hiroshi Nakamura, Reiko Nakashima, and Takehisa Iwai

Department of Vascular and Applied Surgery, Tokyo Medical and Dental University, Graduate School, Tokyo, Japan

Objective: There is no consensus of objective information to determine the indication for lower extremity amputation and the lowest amputation level that will heal. In this report, we studied amputation for the lower extremity ischemia.

Objects and Methods: We measured transcutaneous oxygen tension (tcPO₂) in seven patients who had have ischemic disease of the lower extremity, two patients were in Buerger's disease and five patients were in arteriosclerosis obliterans (ASO). Of these patients, in regard to complications, four patients had diabetes mellitus (DM) and two patients underwent hemodialysis because of chronic renal failure. Six patients of them were men and only one patient was woman, mean age was 59 years old (25~76 years old).

Results: Two patients who had Buerger's disease underwent primary amputation. All another five patients who had ASO underwent secondary amputation. Selections of lower extremity amputation levels were above-knee amputations in three patients, below-knee amputations in two patients and toe amputation in two patients. There were incurable infections in two patients.

Conclusions: It is generally said that if patients have had critical limb ischemia without providing hemodynamic improvement, it is necessary for them to be performed lower extremity amputations within 12 months. In concrete terms, segmental Doppler systolic blood pressures are below 50mmHg and tcPO₂ are below 30mmHg. However in this our study, primary healing rate was 75% when the tcPO₂ was above 20mmHg.

015

FETAL DERMAL MESENCHYMAL CELLS POSSES AN ABILITY TO REGENERATE DERMIS IN THE RECONSTITUTION MODEL OF SCID MOUSE

Kazuo Kishi, Hideo Nakajima, and Tatsuo Nakajima

Department of Plastic and Reconstructive Surgery, Keio University, 35, Shinanomachi Shinjuku, Tokyo, Japan 160-8582

Aim: We have shown that the dermal mesenchymal cells play key roles in the mouse fetal regenerative wound healing. However, the evaluation whether fetal dermal mesenchymal cells possess regenerative capacity in the adult circumstances has not been clarified yet. In the present study, we evaluated the dermal regenerative capacity of fetal dermal mesenchymal cells using reconstitution models on the back of scid mice.

Method: Fetal dermal and fascial tissues were obtained from C57bl fetal mice at 17 days gestation. Tissues were dissected under microscope. Epidermis and dermis were separated with dispase. The dermis and fascia were digested with trypsin and cells from each tissue were suspended. Either dermal cells or fascial cells were mixed with epidermal cells, and transplanted in the skin defect 7mm in diameter made on the back of scid mice. The number of cells transplanted was 1×10^7 for each type of cells. Four weeks later, the transplants were histologically examined.

Results: All of the transplants constituted with dermal cells and epidermal cells regenerate almost normal dermal structures, while transplants mixed with fascial cells and epidermal cells did not regenerate dermis, and left scar like tissues.

Conclusion: The regenerative capacity of fetal dermal mesenchymal cells was shown even in the adult circumstances.

**GENERAL SESSION IV
MODERATED BY YOKA YOSHIMURA**

016

A RETROSPECTIVE STUDY OF VACUUM-ASSISTED CLOSURE IN JAPAN

Suguru Miyamura, Hiroto Terashi, Hideyuki Yanagi, Yoriko Tsuji, Tadashi Nomura, Hideaki Nonomura, and Shinya Tahara

Department of Plastic and Reconstructive Surgery, University of Kobe, Japan

Aim: Vacuum-assisted closure (VAC) system, a new method for wound treatment, has been widespread over the world. There are few reports about this treatment in Japan. The purpose of this report is to consider the usefulness of VAC treatment at our department.

Methods: A retrospective review of 24 patients with 27 chronic wounds, which have been treated with VAC, was presented. The following parameters were studied: age, gender, present illness, complications, duration of VAC treatment, and its result.

Results: Eight women and 16 men were presented. The median age was 66.1 years (range, 20-84 years). Eleven of 27 wounds were mediastinitis. Six were pressure ulcers, and nine were other complicated ulcers. Twelve patients had diabetes mellitus, and nine had hypertension. The median duration of VAC treatment was 32.5 days. Twelve wounds healed only with VAC, and nine healed with a combination of VAC and small operation. Six were halfway stopped a VAC treatment due to infection.

Conclusions: Our results suggest that VAC system is an alternative treatment for chronic wounds of elder patients with high risk.

References:

1. Morykwas M, Argenta LC, Shelton-Brown EI, McGuirt W. Vacuum-assisted closure. A new method for wound control and treatment: animal studies and basic foundation. *Ann Plast Surg* 1997; 38(6): 553-562.
2. Argenta LC, Morykwas M. Vacuum-assisted closure. A new method for wound control and treatment: clinical experience. *Ann Plast Surg* 1997; 38(6): 563-576.

017

CHEMICAL ANALYSIS OF THE SURFACE OF SILICONE MODIFIED BY OXYGEN PLASMA TREATMENT FOR CELL ADHESION

Tai Yamada, Ikuko Ohsugi, Yoshikazu Inoue, Kiyoyuki Mizuno, Takayuki Okumoto, and Yohko Yoshimura

Plastic and Reconstructive Surgery, FUJITA Health University 1-98 Dengakugakubo Kutukake, Toyoake, Aichi, JAPAN

Aim: As we confirmed O₂ plasma treatment is useful for cell adhesion and growth on silicone surface by the culture experiment, reported in the other paper (Ohsugi et al). We aimed to confirm the change of the surface of silicone by O₂ plasma treatment from the material-scientific view.

Material: All materials were cut from a medical grade silicone mammary implant as the same sized disks. Group 1 is silicone disks with no treatment on material's surface. Group 2 is silicone disks with surface modifications by O₂ plasma treatment for 3 minutes. Group 3 is silicone disks with surface modifications by O₂ plasma treatment for 10 minutes.

Method: We investigated each group by the scanning electron microscope (SEM), the atomic force microscope (AFM), and X-ray photoelectron spectroscopy (XPS).

Result: As SEM and AFM shows, after O₂ plasma treatment for 3 minutes, an original inequality formation (10 μm in width) on a surface of silicone were made flat, and for 10 minutes a crack formation occurred.

From investigation by XPS, after O₂ plasma treatment for 3 minutes, the ratio of oxygen on the surface of silicone increased. This phenomenon was thought that hydrophilic functional groups (-OH, -COOH, etc.) were grafted on the surface of silicone. We supposed this is the reason why cell adhesion and growth became more effective.

018

EFFECT OF OXYGEN PLASMA TREATMENT FOR CELL ADHESION PROPERTIES ON SILICONE SURFACE

Ikuko Ohsugi, Tai Yamada, Yoshikazu Inoue, Kiyoyuki Mizuno, Takayuki Okumoto, and Yohko Yoshimura
Plastic and Reconstructive Surgery, FUJITA Health University 1-98 Dengakugakubo, Kutsukake, Toyoake, Aichi, JAPAN

Aim: The silicone polymer is known to be useful and safety as a material of medical implants. But fundamentally its biocompatibility and biostability are questionable. Thus we try to make a hybrid-type silicone implant coating by cell layers. At first, we aimed to confirm cell adhesion and growth on silicone surface.

Material: Group 1 is silicone implants for the augmentation rhinoplasty with no treatment. Group 2 is same type silicone implants with surface modifications by O₂ plasma treatment (3 min). Group 3 is the group 2 implants with serum and plasma coating (20 min).

Method: We co-cultured materials of each group with human fibroblast (2.4*10⁴ cells/ml), for 17 hours, with 5%CO₂, in the temperatures of 37 °C.

Results: Group 1; none of fibroblasts could take the surface of materials.

Group 2; several fibroblasts were found on the surface of those.

Group 3; much more fibroblasts were found than group 2

This result means that the surface modification by O₂ plasma treatment itself enables the cell adhesion and growth on silicone surface.

019

COMPARISON OF BACTERIA-RETAINING ABILITY OF WOUND DRESSINGS

Masahiro Tachi, Shinichi Hirabayashi, Yoshiyuki Yonehara, Gentaro Uchida, and Takuya Tohyama

Department of Plastic and Reconstructive Surgery, Teikyo University School of Medicine, Tokyo, Japan

We studied the bacterial retention capacity of alginate and carboxymethylcellulose dressings, using an infected skin ulcer model on the backs of rats. Wound surfaces were inoculated with either *Staphylococcus aureus* or *Pseudomonas aeruginosa* at a concentration of 1.5×10⁸ cfu/wound. Carboxymethylcellulose dressing, alginate dressing A and alginate dressing B were applied to the contaminated wounds for 12 hours. Each dressing was then divided into two piece. Total viable bacterial count within the dressing was calculated using one piece, and bacterial count released from the dressing into physiological saline was determined using the other piece, enabling bacterial retention rate to be calculated. Each dressing was tested on each of 10 wounds. Statistical analyses were performed using one-way analysis of variance (ANOVA) for replicated measures combined with Duncan's Multiple Comparison Test. Carboxymethylcellulose dressing was most effective in its ability to retain both *Staphylococcus aureus* and *Pseudomonas aeruginosa* (p < 0.05).

Conclusions: The bacterial retaining ability of carboxymethylcellulose dressing was found to be significantly higher than that of alginate dressings in an infected animal wound model.

**GENERAL SESSION V
MODERATED BY YASHIHIKO MAEHARA**

020

REDUCTION OF ISCHEMIA-REPERFUSION INJURY TO SKIN FLAPS BY MONOCLONAL ANTIBODY TO LEUKOCYTE ENDOTHELIAL ADHESION MOLECULE-1

Yasuyoshi Tosa, H. Muramatsu, H. Hukushiyama, T. Otsuka K. Satoh, and Y. Hosaka
Department of Plastic Surgery, Showa University School of Medicine, Shinagawa-ku, Tokyo 142-8666, Japan

Aim: Ischemia-reperfusion (I-R) injury continues to be a problem for successful free tissue transfer and replantation after prolonged periods of ischemia. It has been shown that leukocytes and vascular endothelial cells release a variety of inflammatory mediators during reperfusion after ischemia. The leukocyte endothelial adhesion molecule-1 (LECAM-1) is a ligand on the endothelium for some of the adhesion receptors on leukocytes. The purpose of this study was to evaluate the blockage of leukocyte-endothelial adhesion by a monoclonal antibody (MAb) to LECAM-1 in skin flaps to prevent I-R injury in rats.

Methods: Male SD rats (225-250 g) were used. A skin flap (45×30 mm) supplied by the superficial epigastric A&V including the femoral vessels was isolated unilaterally. The femoral vessels were cross-clamped the epigastric vessels for 9 hours of ischemia. Animals in treated group received MAb to LECAM-1 i.v. 15 minutes prior to reperfusion; those in the control group received normal saline. Skin flap viability was assessed by tracing the outline of viable and nonviable areas. Data were collected for the following 7 days. These data were corroborated with histological evidence on comparable areas of the flap.

Results: Tracing analysis revealed average flap survival area of about 90% in treated group and about 20% in control group (p < 0.05). Histopathologically, few inflammatory changes could be observed in treated group, while marked damage was observed in control group.

Conclusions: From this study, we concluded that treating skin flaps with Mab to LECAM-1 was effective for I-R injury after 9 hours of warm ischemia.

021

CHITOSAN SPONGE WITH PHOTOCROSSLINKABLE CHITOSAN HYDROGEL STIMULATES LARGE AND IMPAIRED WOUND HEALING IN RATS

Toshiaki Ishizuka, Masayuki Ishihara, and Kiyohaya Obara

Department of Medical Engineering and Division of Biomedical Engineering, National Defense Medical College, Tokorozawa, Saitama, JAPAN

Aim: We have previously reported that a photocrosslinkable chitosan hydrogel (Az-CH-LA) has induced wound healing in diabetic mice. However, deep and large skin wounds may be not enough covered by Az-CH-LA only. Therefore, we evaluated whether chitosan sponge with Az-CH-LA accelerates large and impaired wound healing.

Methods: Full thickness-square wounds (25×25 mm) were prepared on the upper back of twenty-four male Sprague Dawley rats. Chitosan sponge and/or Az-CH-LA aqueous solution (30mg/ml) was added onto the wound of each rat and was then irradiated with UV light. Thus, the following four experimental groups were studied: Group (G) 1: control, G2: Az-CH-LA solution only, G3: Chitosan sponge + 0.9% saline, or G4: Chitosan sponge + Az-CH-LA solution. The changes in wound area were measured at day 2, 4, 8, and 16. The skin including the wound was removed from each rat for histological examination.

Results: The wound area in G4 at day 2, 4, and 8 was significantly smaller than the other groups. The number of neovessels and the thickness of granulation tissue in G4 at day 2 and 4 were significantly higher than the other groups.

Conclusions: The chitosan sponge with Az-CH-LA has been found to be more effective in large and impaired wound healing compared with a Az-CH-LA solution only.

022

LIVER REGENERATION AFTER RESECTION OF MULTIPLE LIVER CYSTS

Taketoshi Suehiro, Teijirou Hirashita, Koujirou Mashino, Yasushi Sumiyoshi, Hideya Takeuchi, Masanori Aramaki, Akihiro Watanabe, and Tadashi Kanoh
Department of Surgery, Oita Prefectural Hospital

Background: A regenerative function is in one of the functions of liver, and if it is a normal liver, it will reproduce even to capacity almost before an operation within one year after excision. However, liver substance is pressed over the long period of time, and, as for liver cysts, a postoperative liver reproduction process is unknown. Comparison examination of the multiple liver cysts postoperative liver reproduction was carried out with after the operation.

Patients: It was aimed at one example of a multiple liver cysts operation and three metastatic live tumor (two extended left lobectomy, one right lobectomy) in this hospital. Liver volume was measured before an operation and 14 postoperative days CT, and comparison examination of the standard liver volume ratio called for from height weight was carried out.

Results: Before an operation of liver cysts and 14 postoperative day, liver volume was 151% 45 or 69% in 450 or 680 ml. Before an operation of control group and day 14 postoperative day liver volume was an average of 128% at 116 and 128 or 140% in 62, 72, 56, 71, and 49 or 68% at 618, 720, 532, 680, and 516 or 720 ml, respectively.

Conclusion: Multiple liver cysts postoperative liver reproduction was promoted compared with after the liver resection.

GENERAL SESSION VI MODERATED BY NOBUTOSHI ANDOU

026

LUNG VITAMIN E DEPLETION RESULTING FROM BURN AND SMOKE INHALATION IN SHEEP

Katsumi Shimoda¹, Kazutaka Soejima¹, Tsukasa Isago², Motohiro Nozaki², and Daniel L. Traber¹

Department of Anesthesiology, University of Texas Medical Branch and Shriners' Burn Hospital¹,

Department of Plastic and Reconstructive Surgery, Tokyo Women's Medical University²

Introduction: Oxidants are involved in the pathogenesis of multiple trauma caused by burn and smoke inhalation. α - and γ -tocopherol are major tissue antioxidants and their depletion reflects oxidant injury. We hypothesized that lung vitamin E levels in sheep would be depleted with multiple trauma.

Methods: Sheep (n = 31) were surgically prepared for chronic study, then received either a 40% body surface area third-degree burn, 48 breaths of cotton smoke, a combination of these injuries (B&S) or were given sham injuries. All sheep were resuscitated with Ringer's lactate solution (4 ml/kg/%BSA burn) and mechanically ventilated. The sheep were sacrificed at various time intervals as indicated and vitamin E was measured (nmol/g) in snap-frozen lung samples obtained at autopsy 48 h postinjury.

Results: Lung α -tocopherol was significantly depressed in sheep with the combination injury, smoke alone and burn alone, respectively, compared to sham treated ($p < 0.05$). Lung γ -tocopherol was significantly depressed in sheep with the combination injury compared to sham treated ($p < 0.05$). Neither smoke alone nor burn alone animals were statistically different from sham.

Conclusion: α -tocopherol depletion may be a result of lipid peroxidation, while γ -tocopherol depletion may reflect both lipid peroxidation and peroxynitrite damage (nitro- γ -tocopherol formation). These results suggest that animals receiving a combination burn and inhalation injury have undergone marked oxidative stress.

027

COMPARATIVE STUDY OF NONINVASIVE VASCULAR IMAGING TECHNIQUES IN THE EVALUATION OF CHRONIC VENOUS LEG ULCERS

Takashi Yamaki and Motohiro Nozaki

Department of Plastic and Reconstructive Surgery, Tokyo Women's Medical University, Tokyo, Japan

Aim: To compare noninvasive vascular imaging techniques in the evaluation of chronic venous leg ulcers, and to detect the discriminating parameters between primary valvular incompetence (PVI) and postthrombotic syndrome (PTS).

Methods: A total of 61 limbs with chronic venous ulcers were included in this study. Of 61 limbs, 31 had PVI and remaining 30 had documented PTS. The distribution of venous reflux including superficial, deep, and perforating vein was determined by duplex ultrasound. The venous functions including venous volume (VV: ml), venous filling index (VFI: ml/sec), ejection fraction (EF: %), and residual volume fraction (RVF: %) were evaluated by air plethysmography (APG). The near-infrared spectroscopy (NIRS) was used to measure calf muscle total hemoglobin (tHb), oxygenated hemoglobin (O_2Hb), and deoxygenated hemoglobin (HHb) levels using APG exercise protocol, and the changes in these parameters were divided into three patterns; Type I: The HHb does not increase beyond the O_2Hb during the total procedure. Type II: The HHb increases beyond the O_2Hb during the excise. Type III: The HHb increases beyond the O_2Hb during the total procedure. Patients with Type III were considered to have most significant calf muscle dysfunction.

Results: Superficial venous incompetence was significantly predominant in the PVI ($p = 0.031$). The proportion of deep vein incompetence was significantly higher in the PTS ($p = 0.023$). Of these, patients with PTS had significantly higher proportion of femoral vein (FV) and popliteal vein (POPV) incompetence compared to these with PVI ($p = 0.0006$, 0.0015, respectively). There was no statistically significant difference in the proportion of perforator incompetence between the groups ($p = 0.611$). There were no significant differences in VV (94.8 ± 43.7 , 84.7 ± 48.8 , $p > 0.999$), VFI (5.04 ± 2.49 , 5.19 ± 3.15 , $p = 0.236$), EF (51.9 ± 19.2 , 47.2 ± 25.3 , $p = 0.903$), and RVF (54.8 ± 22.3 , 66.1 ± 29.8 , $p = 0.378$). In the NIRS examinations, Type I was found in 20 limbs (64.5%) in the PVI and 3 (10%) in the PTS, and this was a statistically significant ($p < 0.0001$). On the contrary, Type III was seen in 1 limb (3.2%) in the PVI and 19 (63.3%) in the PTS. The proportion of Type III was significantly higher in the PTS ($p < 0.0001$).

Conclusions: These data suggest that reflux in the FV and POPV might play more important role in the development of venous leg ulcers in patients with PTS. The APG-derived parameters did not improve the discrimination power between the two groups. The increased NIRS-derived HHb in the exercising calf muscle is profound in patients with PTS, suggestive of a promising parameter both in the discrimination of the patients with chronic venous leg ulcers and in the follow-up of the patients with deep vein thrombosis.

GENERAL SESSION VII MODERATED BY CHIFUMI YOSHIDA 030

A CASE OF ULCERATIVE COLITIS WITH PERISTOMAL PYODERMA GANGRENOSUM

Tomoe Ebata

Department of Nursing, Chiba University Hospital

Introduction: Pyoderma gangrenosum is a skin disease that can result in progressive necrosis and formation of painful ulcers. This is to report on a case where the patient developed the disease around a stoma.

Case: A 40-year-old female patient developed ulcerative colitis (UC) when she was 25. At the age of 38, the exacerbation of UC required a colectomy and ileal pouch anal canal anastomosis (IACA), following which, a permanent ileostomy was constructed due to pouchitis. In April 2003, she developed redness around the stoma with pain, which resulted in the gradual formation of an ulcer. She was hospitalized with a diagnosis of peristomal pyoderma gangrenosum (PPG). After admission to the hospital, the dosage of predonine was increased to 30 mg/day, and dapsone was concomitantly administered. Clobetasol propionate and sulfadiazine silver were topically administered 1-4 times daily. The wound region adjacent to the stoma was protected with a gauze dressing. Although the gauze was changed each time, it was contaminated with fecal matter, the suffering thereby inflicted on the patient was too severe due to the associated smell and staining of her clothing. For this reason, she alternatively started wearing a stoma bag, which enabled periodic replacement once daily. Following a gradual improvement of the ulcer, she was discharged from the hospital two months after admission. Her recovery was confirmed four months after onset.

Conclusion: PPG is accompanied by pain, and treatment also involves suffering by the patient. We need to make the diagnosis and determine the treatment method while the disease is still in its early stages. The selection of the method of care should be made with consideration of the practical aspect of the patient's defecation control.

031

IMPORTANT FACTORS IN THE HEALING OF A PRESSURE ULCER IN THE ISCHIUM OF A SPINAL CORD-INJURY PATIENT WHOSE PRESSURE ULCERS RECURRED OVER 26 YEARSAyumi Sugai¹ and Hiromasa Yamamoto²Department of Nursing, Hoshigaoka Koseinenkin Hospital¹Department of Orthopedic Surgery, Hoshigaoka Koseinenkin Hospital²

Objectives: Pressure ulcers in the ischium of spinal cord-injury patients are caused by their wheelchair-bound lifestyle. Such injuries are intractable and limit a patient's activities of daily life for long periods. Here, we report our experience with a patient whose pressure ulcer was healed without limitation of her daily activities.

Subject: A woman aged 56, who had injured her thoracic spinal cord 26 years earlier, developed a pressure ulcer in the right ischium. The injury healed and then recurred over 10 admissions for treatment, and the maximum period over which the patient stayed at home without a pressure ulcer was only two months over the past several decades. In June 2002, she was healed and discharged, but the pressure ulcer recurred in the same region only one month later.

Course: She was treated in the outpatient department, but the affected area expanded and the condition aggravated and invaded the subcutaneous tissue. The causes were considered to be a body posture leaning to the right, rubbing during decompression, and pressure on the lesion exerted by a cushion of the wheelchair. Treatment of the patient began from a consideration of daily activities, including work and housekeeping. The cushion was changed to a new one that helped the patient maintain a good posture and prevented rubbing. In addition, the patient was instructed to develop a decompressing action that dispersed the body pressure to the posterior thigh, and was shown how to sit during monthly visits to the outpatient department. The affected lesion was washed every day in the shower, and was treated with sugar and povidone-iodine.

Results: The patient observed changes in the affected lesion of the pressure ulcer, and reported changes that occurred in her daily life. In April 2003, the pressure ulcer had healed and has yet to recur 8 months later.

Conclusions: (1) For pressure ulcers in the ischium of a spinal cord-injury patient, it is of importance for healing and prevention of recurrence to find the cause of the pressure and to improve the condition, rather than to treat the affected lesion. (2) Instructions regarding adoption of the correct sitting position, i.e., a major factor in daily life, and continuous care intervention improved the patient's self-care ability.

**SYMPOSIUM I-1. PATHOPHYSIOLOGY AND TREATMENT OF PRESSURE ULCER
MODERATED BY KOICKI HIRATA**

SI-01

THE STUDY OF CLINICAL USEFULNESS OF BASIC FGF

Masahiro Hamanoue, Tetuhiro Nakajo, Fumio Kijima, Hiro Nisijima, and Sigeho Maenohara

Department of Surgery, Kagoshima Kouseiren Hospital, Kagoshima, Japan

Recently, basic FGF (fibroblast growth factor) has been made clinical use for wound cure. We studied clinical usefulness of basic FGF.

Cases: Nine cases took wound necrosis by infection. Two cases took burn after mastectomy. One case took abscess in pelvic space.

Methods: We investigated healing rate of wound by healing area per day. And we evaluated histological wound healing by HE staining, labeling index of Ki64 staining, CD34 staining for index of neovascularization, and α -smooth muscle actin staining for differentiation of fibroblasts.

Result: Healing rate of nine cases with wound necrosis by infection was 5 cm²/day and that of two cases with burn after mastectomy was 0.5 cm²/day. Labeling index was more than 10%. CD34 staining showed increasing neovascularization of healing tissues with treatment of bFGF, compared with that received other treatments. But the case with abscess did not recover and took the reoperation for treatment.

Conclusion: We concluded that bFGF was efficacious against tissue damage and useful for wound cure. But basic FGF was not useful for the infectious focus without control.

SI-02

LPA-AND PDGF-STIMULATED CONTRACTION OF COLLAGEN MATRICES INVOLVES NEITHER BY MYOSIN LIGHT CHAIN PHOSPHORYLATION NOR BY MLC KINASE

M Abe, O Ishikawa, C-C Ho*, and F Grinnell*

Department of Dermatology, Gunma University Graduate School of Medicine

*Department of Cell Biology, University of Texas Southwestern Medical Center

Fibroblast-collagen matrix contraction has been used as a model system to study how cells organize connective tissue. Previous work showed that lysophosphatidic acid (LPA)-stimulated collagen matrix contraction is independent of Rho kinase whereas platelet-derived growth factor (PDGF)-stimulated contraction is Rho kinase dependent. The current studies were carried out to learn more about the molecular motors responsible for LPA- and PDGF-stimulated fibroblast-collagen matrix contraction. Fibroblasts whose MLC kinase was knocked down using siRNA were used to measure matrix contraction in the presence of LPA or PDGF with or without the Rho kinase inhibitor added. The extent of contraction of MLC kinase-silenced cells was not detectably different from control cells. Other experiments were carried out to test the effects of LPA and PDGF on MLC phosphorylation with and without Rho kinase inhibitor. After 15 min of growth factor stimulation, levels of diphosphorylated MLC were highest in cells in LPA-containing medium and lowest in PDGF containing medium. Prior addition of Rho kinase inhibitor markedly reduced phosphorylation in every case. These observations suggested that stimulation of collagen matrix contraction required neither growth factor stimulation of MLC phosphorylation nor MLC kinase.

SI-03

RECOMBINANT G-CSF INDUCES DOWNREGULATION OF IL-12 TRANSCRIPTION AFTER PARTIAL HEPATECTOMY

J Okiyama, K Oishi, K Hayamizu, X Aihaiti, I Ohmori, M Yoshimitsu, T Itamoto, and T Asahara

Department of Surgery, Division of Frontier Medical Science, Graduate School of Biomedical Sciences, Hiroshima University

Aims: Recent studies have demonstrated that administration of recombinant IL-12 induces cytotoxicity against regenerating hepatocytes. We measured transcription levels of IL-12 in the remnant liver in a model of partial hepatectomy (PHx) to elucidate the mechanism of already reported facilitation of liver regeneration by recombinant human G-CSF (rhG-CSF).

Methods: After PHx of F344 rats, total RNA of the remnant liver was extracted and mRNA levels of IL-12p35 and G-CSF were quantified by RT-real time PCR.

Results: G-CSF mRNA levels increased and IL12p35 mRNA levels decreased at 6 and 20 hours after PHx ($P < 0.01$). Pre-PHx rhG-CSF treatment significantly further down-regulated IL12p35 mRNA levels at 20 hours ($P < 0.01$).

Conclusions: This result suggests that rhG-CSF pretreatment accelerates tissue repair of the liver at least partially through regulation of cellular immunity.

References: Egi H, et al. Cytokine 2002;18(3):164-7.
Kitayama T, et al. Transplantation 2003;75(4):553-6.

SI-04

NONINVASIVE APPROACH FOR REFRACTORY ENTEROVAGINAL FISTULA

Nishimori Hidefumi, Fumitake Hata, Tomohisa Furuhashi, Gentaro Ishiyama, and Koichi Hirata

Department of Surgery, Sapporo Medical University School of Medicine, Minami-1, Nishi-16, Chuo-ku, Sapporo 0608543, Japan

Enterovaginal fistula is generally difficult to treat, and a surgical treatment is often chosen. There are many cases that require extended bowel resection for severe adhesion in the pelvic cavity and just as many cases of postoperative complications. The present case involves a 61-year-old female. After radical operation for ovarian cancer, a recurrence was recognized in the pelvic cavity despite chemotherapy. After that, recurrence was found in the pelvic cavity, and low anterior resection combined with the resection of recurrent tumor was performed. Enterovaginal fistula and pelvic fistula were found from the 10th postoperative day; therefore urethral catheter was placed transvaginally. Those fistulas were simplified afterwards, and the tube for percutaneous transhepatic cholangio drainage (PTCD) was placed and then removed after several exchanges. After that, enteric juice was identified for a few days, but the fistula was completely closed, and no recurrence was found.

It is easy for discharge from enterovaginal fistula to increase because of the anatomical position of the fistula; since there is no appropriate device for this fistula, treatment is difficult. It seems that, in selected cases in which the patient heals conservatively without losing quality of life for patients, it is possible to place a tube in an appropriate position under the x-ray and change it step by step.

SI-05

ATTEMPT TO IMPROVE WOUND HEALING CONSIDERING THE TIMING OF SUTURE REMOVAL AND THE DRESSING

Toshihiro Kite¹, Shinya Arinaga¹, Koichi Ishikawa², Norio Shiraiishi², and Seigo Kitano²

Department of Surgery, Takada Chuo Hospital¹, and First Department of Surgery, Oita Medical University²

Introduction: In the surgical field, "suture removal on 7 days after surgery" and "daily disinfection and coverage with gauze" have been customs. The timing of suture removal and the postoperative wound treatment were devised, and their effect on wound healing was compared with the conventional method.

Method: The abdominal incision was divided into two parts, one half of the suture was removed on 3 days after surgery, the other half of the suture was removed on 7 days after surgery, and the conditions of the wound were compared between 7 and 14 days after surgery. In addition, the skin was disinfected only immediately before incision, and the closed dressing was given on the wound.

Results: There were no differences in the degree of skin approximation, the degree of redness, subcutaneous swelling, pain and postoperative scar between both days. However, the suture removal on 3 days gave no scar of suture, which was better cosmetically. The frequency of treatments also decreased, which led to cost reduction.

Discussion: Since the wound is covered with epithelial cells in 48 hours, the suture removal on 3 days and the closed dressing are desirable in the abdominal incision to which no especially strong stress is added from the viewpoint of wound healing. In addition, this method is better than the conventional method from the aspect of cosmetics and cost reduction. We will investigate this method further in the future.

SYMPOSIUM I-2. PATHOPHYSIOLOGY AND TREATMENT OF PRESSURE ULCER

Moderated by Toshimasa Asahara

SI-06

STERILIZED ACELLULAR ALLOGENEIC DERMAL MATRIX FOR SCAFFOLD OF CULTURED COMPOSITE SKIN

Jingping Guo, Yoshihiro Takami, Ryo Yamaguchi*, Noriko Shiota, Ken Ogo, and Kiyonori Harii

Department of Plastic Surgery, Kyorin University School of Medicine, *BCS, Inc.

Aim: Acellular Allogeneic Dermal Matrix ADM has been used for scaffold of cultured composite skin. However, possibility of disease transmission by ADM remains as its potential risk. So it is important to reduce the risk by ADM transplantation the purpose of this study was to evaluate whether ADM could be used as a scaffold of cultured composite skin safely.

Method: Four different kinds of methods were used for sterilization, such as 98% glycerol, Pasteurization, Gamma irradiation, and ethylene oxide gas sterilization (EOG). Histological, immunohistochemical and electrophoretic (SDS-PAGE) properties were evaluated. The property as a scaffold of cultured composites skin was evaluated using normal human cultured keratinocytes.

Results: Four methods did not induce apparent collagen degradation of ADM on SDS-PAGE. However, damage of basement membrane structure was observed by ADM treated with Pasteurization, Gamma irradiation, and EOG histological. These three methods also resulted in the poor regeneration of the epidermal layer in cultured composite skin.

Conclusion: The results suggested that glycerol treatment was found to be the best method of ADM sterilization as far as its scaffold was concerned.

SI-07

REGULATION OF VEGF-INDUCED ANGIOGENESIS BY MMPs

Takayuki Shiomii¹, Gakuji Hashimoto¹, Isao Inoki¹, Yutaka Fujii², Eiji Ikeda¹, and Yasunori Okada¹

¹Department of Pathology, School of Medicine, Keio University, Tokyo, Japan

²Department of Chemistry, Fukui Medical University, Fukui, Japan

Aim: We have recently reported that connective tissue growth factor (CTGF) inhibits the angiogenic activity of VEGF₁₆₅, one of the vascular endothelial growth factor (VEGF) isoforms, through the complex formation with VEGF₁₆₅. In the present study, we examined the susceptibility of the VEGF₁₆₅/CTGF complex to matrix metalloproteinases (MMPs), ADAMTS4 and serine proteinases, and evaluated the recovery of the angiogenic activity of VEGF₁₆₅ after the treatment.

Methods: VEGF₁₆₅/CTGF complex was digested with six different MMPs, ADAMTS4, elastase or plasmin. The reaction products were analyzed on silver-stained gels and by immunoblotting. Angiogenic activity of the complex treated with MMPs was assayed by in vitro tube formation assay and in vivo angiogenesis assay using a Matrigel injection model in mice.

Results: Among the MMPs, MMP-1, -3, -7, and -13 processed CTGF of the complex into the major NH₂- and COOH-terminal fragments, whereas VEGF₁₆₅ was completely resistant to the MMPs. The in vitro angiogenic activity of VEGF₁₆₅ blocked in the VEGF₁₆₅/CTGF complex was reactivated to original levels after CTGF digestion of the complex with MMPs. Recovery of activity was further confirmed by in vivo angiogenesis assay.

Conclusions: These results demonstrate that CTGF is a substrate of MMPs and that the angiogenic activity of VEGF₁₆₅ suppressed by the complex formation with CTGF is recovered through the selective degradation of CTGF by MMPs. MMPs may play a novel role through CTGF degradation in VEGF-induced angiogenesis during formation of granulation and scar tissue in wound healing process.

SI-08

SKIN REGENERATION BY HUMAN MESENCHYMAL STEM CELLS AND BASIC FIBROBLAST GROWTH FACTOR

H Nakagawa, K Akino, and S Akita
Department of Developmental and Reconstructive Medicine, Graduate School of Medical and Dental Sciences, Nagasaki University, Nagasaki, Japan

Skin and soft tissue defects are sometimes problematic especially when the defects large, contaminated, irradiated, or poor blood supplied. The human mesenchymal stem cells (hMSCs) are proliferated upon basic fibroblast growth factor (bFGF) stimuli in vitro and in vivo. In this experiment, the skin and soft tissue defects are investigated if the wounds are able to be reepithelialized or accelerated by hMSCs, bFGF and porcine-derived bilayered skin template.

1.5×1.5 cm² nude rat skin and soft tissue defects including panniculus carnosus are excised and 1×10⁶ hMSCs and various doses of bFGF (1–100 µg) applied. Before and after normal reepithelialization, the tissues are tested for protein expressions by immunohistochemistry and Western blotting.

The wound sizes are significantly decreased at day 7 with hMSCs with 1, 10, or 100 µg bFGF compared to hMSCs-alone or medium-only. All the wounds healed by day 42. 42 Kda and 38 Kda human-derived pancytokeratin expressions, which do not cross-react with murine antigens, by Western blotting significantly augmented by 10 µg bFGF compared to hMSCs-alone. The epidermal immunolocalizations such as integrin α3 and SKALP (Skin-derived Anti Leukoproteinase) are greatly elevated in time and dose-dependent manner. Human pan-cytokeratin expressions are immunoreactive even at day 42.

These data suggest the skin and soft tissue wound healing is accelerated by hMSCs together with bFGF, partly by means of differentiation of hMSCs toward epidermal components.

SI-09

DISPERSION OF PRESSURE TO THE HEAD IN BRAIN/NEUROSURGERY

Chika Anzai, Kaoru Kosidawa, Rumi Sanpei, and Masayuki Kouda
Itabashi Chuo General Hospital

Introduction: In this field, there are limitations to the positioning of the head in accordance to the postsurgery drain control and respective disorders. Dispersion of pressure to the head is attempted.

Methodology: Items used: air mat, pressure reducing mat, water-filled pillow, bath towel folded into quarters, pressure gauge

- (1) Experiment subject: 14 patients with no self bodily movements, stable in bed, 14 points or more on the Braden scale at 30 degrees
- (2) Procedure: After application of each pressure reducing device on the experiment subject (laying horizontally in the supine position), measure the pressure to the head three times and compute the average value

Results: Pressure to the head averaged 53.9 mmHg for the towel, mat(s) only. The water-filled pillow was lower than the others, at 48 mmHg.

Examination: According to Sanada "in the attempt to lower the pressure to the body, either the weight is lightened, or the area subject to pressure is expanded." The back of the head, which is an area highly susceptible to pressure sores for the head, the area subject to pressure is confined. It could be said that the conditions for the subjects in this experiment were highly susceptible to pressure sores. Therefore, the frequent transferring of pressure, the use of air mats and water-filled pillows had been considered as prevention measures; however, with this experiment, the effectiveness of water-filled pillows was confirmed.

It is viewed that since pressure to the head depends on individual body weight and overall body conditions, at the prevention stage, it is critical to select the pressure reducing device in accordance to the evaluation and assessment.

SYMPOSIUM I-2. PATHOPHYSIOLOGY AND TREATMENT OF PRESSURE ULCER

Moderated by Hiroyuki Kuwano

SI-10

ADMINISTRATION OF BASIC FIBROBLAST GROWTH FACTOR bFGF FOR INTRACTABLE RECTOVESICAL FISTULA — A CASE REPORT

Ryoichi Onozato, Satoru Yamaguchi, Takayuki Asao, Tsutomu Kobayashi, Soichi Tsutsumi, and Hiroyuki Kuwano

Department of General Surgical Science, Gunma University Graduate School, Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, 371-8511, Japan

Case: 62-year-old female. Drainage of abscess and hysterectomy were performed for pelvic peritonitis. But stool-like exudates was observed at postoperative 2 days. Transverse colostomy and drainage of the abscess were performed. Transvaginal closure of the vaginal stump was performed for rectovaginal fistula. After a while, intractable rectovesical fistula was newly observed.

Clinical course: Because patient was contracted articular rheumatism, she wanted to avoid operation. Under sufficient informed consent, we started the treatment of injection with bFGF via the drainage tube to rectovesical fistula (50 µg/day). Narrowness of the fistula was checked by fistulography, and decrease of eduction of stool like exudates was observed. The fistula was closed after 2.5 months from beginning of the treatment.

Summary: We reported a case that bFGF was available for intractable nontumorous enterogenic fistula.

SI-11

BASIC FIBROBLAST GROWTH FACTOR ACCELERATES APOPTOSIS IN ACUTE INCISIONAL WOUND HEALING AND REDUCES SCAR FORMATION

Yoshikiyo Akasaka¹, Ichiro Ono², Toshiharu Yamashita², Kowichi Jimbow², and Toshiharu Ishii¹

¹Second Department of Pathology, School of Medicine, Toho University, Tokyo, Japan

²Department of Dermatology, Sapporo Medical University School of Medicine, Sapporo, Japan^b

Apoptosis has been shown to play an important role in the regulation of wound healing, and growth factors can mediate this process. In this study, we examined the relationship between the degree of healing and the level of apoptosis in full-thickness-incisional skin wounds, which were treated by conventional suturing with or without intradermal injection of bFGF (0.1 µg and 1 µg/cm of wound). The width of wound tissue showed that the degree of granulation formation in the 1 µg-bFGF-treated group significantly increased on day 7, whereas the degree of scar formation significantly decreased on days 14 and 28. Similarly, apoptotic cells significantly increased in the number on day 4 in the 1 µg-bFGF-treated group compared with that of the control group ($p = 0.024$), and decreased on days 14 and 28. These findings therefore, suggest that the accelerated apoptosis in the bFGF-treated wounds contributes to the decreased cellularity in inflammatory change through elimination of cells with apoptosis, which resulted also in the reduction of scar formation. It therefore hypothesized that apoptosis is involved in the maturation of an acute wound into scar formation, and that bFGF can accelerate this process.

SI-12

INTERACTION BETWEEN HUMAN MESENCHYMAL STEM CELLS AND BASIC FIBROBLAST GROWTH FACTOR OR OTHER CELL TYPES

K Akino, T Mineta, H Nakagawa, and S Akita

Department of Developmental and Reconstructive Medicine, Graduate School of Medical and Dental Sciences, Nagasaki University, Nagasaki, Japan

The basic fibroblast growth factor (bFGF) is known to proliferate and maintain the adult stem cells. The human mesenchymal stem cells (hMSCs) are very homogenous and constitute of euchromatin nuclei in 10% fetal bovine serum (FBS) and induce the heterochromatin nuclei by bone morphogenetic protein.

In order to further investigate other growth factor, other cell types like bFGF, keratinocytes, dermal fibroblasts and endothelial cells, the cellular and molecular analyses are performed in vitro assay systems.

The various doses of bFGF (0.25–25 µg/ml) significantly proliferate the hMSCs in time-dependent manner in a serum-free medium. The ultra-structure by electron microscopy revealed the very small, round and immature both cytoplasmic and nuclear structure resembling the 10% FBS-cultured cells. The hMSCs and bFGF and other cells are co-cultured in 8 µm-pore chamber systems for 16-hour incubation. The hMSCs successfully migrate through the pore when keratinocytes, dermal fibroblasts, endothelial cells or bFGF (345.0 ± 68.86, 63.8 ± 16.50, 36.2 ± 9.60, 12.0 ± 4.89, $p < 0.01$) are placed lower chamber. The clear cellular attachment between hMSCs and keratinocytes is observed in the monolayer co-culture by electron microscopy.

The hMSCs are stimulated by bFGF and interact with other cell types such as keratinocytes, dermal fibroblasts and endothelial cells in vitro.

SI-13

OPEN WOUND INTEGRATED REGENERATIVE THERAPY WITH TOPICAL BFGF TRAFERMIN FOR DEEP SOFT TISSUE DEFECTS WITH BONE EXPOSURE

N Ohura, S Ichioka, T Asano, and T Nakatsuka

Department of Plastic and Reconstructive Surgery, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama, Japan

Aim: Recently advocated concept of wound bed preparation (WBP) has systematically concreted the critical components of wound care, including debridement, bacterial balance, and management of exudate. We succeed in experiences by topical pharmaceutical therapy combined with WBP. We aimed to demonstrate availability of the wound management.

Methods: Since 1999, 18 exteriorized-bone patients with deep soft tissue defects were treated with this treatment. Our procedure was composed of four steps; 1) Resection or debridement, 2) Wound cleansing (soaking foot and hand in the hot bath with antibacterial carbonated agent), 3) Topical medication: basic fibroblast growth factor (Trafermin; KAKEN, Tokyo) and bucladine sodium (Actocin ointment; Daiichi Pharmaceu. Co. Ltd, Tokyo), and 4) Dressing.

Results: The average time for whole coverage of the exposed-bone area with healthy granulation was 23 ± 13 (median:19, range: 7–47) days after surgical treatment (the 1st step). The average time for complete epithelization was 72 ± 42 (55, 26–150) days in the patients who had not undergone skin graft. For minor amputations of diabetic feet the healing time until complete epithelization was 65 ± 46 (44, 26–150) days on an average.

Conclusions: We concluded that the integrated approach mobilizing commercial available products for wound healing under optimally prepared environment offers advantage of facilitating the efficacy of exogenous therapeutic measures on condition that endogenous regeneration has been accelerated.

SI-14

MODIFICATION OF WOUND HEALING PROCESS USING BASIC FIBROBLAST GROWTH FACTOR

Ichiro Ono¹, Toshiharu Yamashita¹, Akihiro Tominaga¹, Yoshiharu Akasaka², Toshiharu Ishii², Kowichi Jimbow¹

¹Department of Dermatology, Sapporo Medical University School of Medicine, ²Second Department of Pathology, Toho University School of Medicine

In order to identify a means to reduce the scar formation of the skin after incision, this study examined the wound healing effect of bFGF in humans. bFGF was administered at dose of 0.1 and 1 µg per cm of sutured immediately after an operation. The drug was injected once into the dermis of the margins of wounds using a 27 G needle attached to a 1-ml syringe to the patients. The lengths of the treated wounds varied from 1.5 cm to 23 cm, and the subjects were 2 to 76 years old. Sutured wounds after excision of skin tumors from the face, trunk and limbs and the sutured wounds such as those at the donor sites of full thickness skin grafts were treated with low dose bFGF injections. Postoperative administration of bFGF, inhibited scarring and accelerated healing without any serious side effects. Although double-blind studies are needed, we expect that so-called scar-less surgery may be possible by establishing of more sophisticated methods for administering bFGF and its combination with other drugs and/or gene therapy in the future.

Keywords: Wound healing, clinical evaluation, acute incisional wound, scar-less surgery

SYMPOSIUM I-2. PATHOPHYSIOLOGY AND TREATMENT OF PRESSURE ULCER

Moderated by Takashi Tajiri

SI-15

WOUND BED REGENERATION BY AUTOLOGOUS UNFRACTIONATED BONE MARROW TRANSPLANTATION

Sachio Kouraba¹, Masato Yasuda¹, Masanobu Kumakiri¹, Taisuke Sakamoto², and Tsuneki Sugihara²

¹Department of Dermatology, School of Medicine, Fukui University, Matsuoka

²Department of Plastic and Reconstructive Surgery, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

Although the pathogenesis of chronic ulcer still remains undefined, one of the characteristics is poor granulation of wound bed, in other words, disruption of wound angiogenesis. Experimental studies have shown that bone marrow (BM) derived endothelial progenitor cells take part in postnatal neovascularization in cutaneous wound healing. BM cells also contain mesenchymal stem/progenitor cells with pluripotency differentiating into myofibroblasts and fibroblasts. We treated nonhealing leg and foot ulcers sustained over one year by topical transplantation of autologous fresh unfractionated BM-impregnated collagen sponge (BMICS). In all patients, the treatments led to rapid generation of well-vascularized granulation tissue. All of the wounds were healed up completely with conservative treatment or skin grafting. We suggest that direct transplantation of autologous bone marrow cells on the wound may represent a novel procedure for regeneration of disrupted wound healing on recalcitrant cutaneous ulcers.

SI-16

ACCELERATION OF WOUND HEALING WITH PDWHF AND CULTURED CELLS

Kazutaka Soejima, Daisuke Fujisawa, Takashi Honda, Tsukasa Isago, and Motohiro Nozaki
Department of Plastic and Reconstructive Surgery, Tokyo Women's Medical University, Tokyo, Japan

Objective: Effects of platelet derived wound healing factor (PDWHF)¹ with or without cultured cells on angiogenesis in treatment of defected wounds using artificial dermis (AD) were investigated in rat experimental model.

Methods: Wistar strain rats were used for this study. The PDWHF was prepared from platelets. The endothelial cells and fibroblasts were prepared from thoracic aorta and back skin respectively. Two sites of 2.5×2.5 cm full-thickness wounds were created on back of the animals. Artificial dermis (TERUDERMIS®, TERUMO Co, Japan) were grafted to the wounds. Prior to the AD grafts, following 4 groups were established, control group: AD alone (n=6), PDWHF group: AD treated with PDWHF (0.1 ml/cm²) (n=6), cultured cells group: disperse of 1×10⁶ cultured endothelial cells and fibroblasts between AD and wound (n=6) and combination group: combination of PDWHF and cultured cells (n=6). Five days after surgery, degree of angiogenesis was evaluated by gross inspection and histological study. Evans blue perfusions test was performed to evaluate the degree of new capillary formation in the generated dermis qualitatively.

Results: The combination group showed vascular invasion into AD 5 days after surgery. In this group, the absorbance of the Evans blue extracted from the grafted dermis was highest among the group.

Conclusion: The result of the present study revealed that treatment with PDWHF combined with cultured endothelial cells and fibroblasts accelerate the wound angiogenesis. This method may be beneficial to shorten the period between the AD grafts and the secondary skin grafting.

References:

1. Krupski WC, Reilly LM, Perez S, et al. A prospective randomized trial of autologous platelet-derived wound healing factors for treatment of chronic nonhealing wounds: a preliminary report. *J Vasc Surg.* 1991;14(4):526-532.

SI-17

VIABILITY AND FUNCTION OF AUTOLOGOUS AND ALLOGENEIC FIBROBLASTS SEEDING IN DERMAL SUBSTITUTES AFTER IMPLANTATION

Naoki Morimoto¹, Shigehiko Suzuki¹, Yasumi Saso¹, Kenji Tomihata², Tsuguyoshi Taira², Yoshitake Takahashi², and Noriyuki Morikawa²

¹Department of Plastic and Reconstructive Surgery, Graduate School of Medicine, Kyoto University, Kyoto City, Japan

²GUNZE Ltd. Research & Development Department, Ayabe City, Japan

Aim: The Aim of this study is to investigate the viability and distribution in vivo of autologous and allogeneic fibroblasts seeded in the collagen sponges after implantation and to clarify which is more effective for wound healing.

Methods: Three full-thickness wounds were created on the backs of guinea pigs and acellular substitute, substitute seeded with autologous fibroblasts at the density of 1.0×10⁶ cells/cm², and substitute seeded with allogeneic fibroblasts at the same density were transplanted. Before implantation, fibroblasts were labeled with PKH26.1, 2, 3 weeks after implantation, the epithelized areas were measured and specimens were taken.

Results: PKH26 labeled autologous and allogeneic fibroblasts remained viable. In the wound covered with autologous fibroblasts seeded substitute, the epithelization was fastest, and wound contraction was minimal. In contrast, in the wound covered with allogeneic fibroblasts, the epithelization was latest and the contraction was largest.

Conclusions: Allogeneic fibroblasts seeded in the collagen sponge took and remained viable on the grafted area, but adversely affected the wound healing.

SI-18

COMPARISON BETWEEN OMENTAL IMPLANTATION AND OMENTAL PATCH FOR PERFORATED ACETIC ACID-INDUCED GASTRIC ULCER IN RATS

Yasunori Matoba, Akira Tokunaga, Yukichi Moriyama, Shinya Iida, Norio Matsukura, Takashi Tajiri, and Hironori Katayama
Center for Digestive Diseases, Nippon Medical School, Kanagawa, Japan

Aim: Omental implantation, a surgical procedure in which a perforated gastric or duodenal ulcer is repaired by drawing and implanting a portion of the omentum into the digestive tract, accelerates ulcer healing and inhibits ulcer recurrence compared with omental patch from clinical results. To clarify these mechanism and differences, we investigated ulcer healing in two groups.

Methods: In two groups of rats in which acetic acid-induced gastric ulcers were perforated. Omental implantation was used for repaired in one group and omental patch was employed in the other group. Basic fibroblast growth factor (bFGF) mRNA-positive cells were identified and localized by in situ hybridization. Fibroblast growth factor receptor (FGFR)-positive cells were identified and localized by immunohistochemical analysis.

Results: Antiinflammatory and angiogenic activity and accelerated collagen synthesis were seen in the omental implantation group. bFGF mRNA-positive cells (macrophages, fibroblasts, and endothelial cells) and FGFR-positive cells were seen within the omentum, resulting in abundant collagen production and rapid epithelial regeneration. In the omental patch group, extensive neutrophilic infiltration and ulcer recurrence were seen. Few bFGF mRNA-positive cells and FGFR-positive cells were seen within the omentum, resulting to inhibit omentum becoming to be granulation tissue and ulcer healing.

Conclusions: These results indicated that omental implantation accelerated ulcer healing, and the presence of bFGF mRNA and FGFR played a significant role in this phenomenon.

SYMPOSIUM II: BASIC RESEARCH AND CLINICAL APPROACH FOR IMPAIRED WOUND HEALING
MODERATED BY TAKASHI NAKAZUKA
S2-01

NEGATIVE-PRESSURE DRESSINGS IN THE TREATMENT OF INFECTED PRESSURE ULCERS

Tsukasa Isago and Motohiro Nozaki

Department of Plastic and Reconstructive Surgery, Tokyo Women's Medical University, Tokyo, Japan

Applying negative pressure to wounds may speed the formation of granulation tissue, decrease the amount of localized edema, increase blood flow, and accelerate healing. In the present study, we treated 10 patients with stage IV chronic pressure ulcers associated with infection by this negative pressure dressings. The aim of this investigation is to study the efficacy of this therapeutic method for pressure ulcers with the infection by this negative pressure dressings. The long (A) and short (B) diameters of ulcer were measured to determine the size, and the vertical distance from the skin to the deepest point of ulcer was measured to determine the depth. Lesions were measured initially, at weekly intervals. The area of the lesion was taken to be 3.14×A/2×B/2 (cm²). In comparison with the area in the start and end of the treatment, the area had been reduced in all cases, and the reduction of 55.1% was shown from the area at the baseline. In comparison with the depth in the start and end of the treatment, the depth also decreased as well as the area in all cases, and the reduction of 61.2% was shown from the depth at the baseline. In view of the period of evaluation, the method is considered markedly effective in reducing the size and depth of ulcer.

References

- 1) Argenta LC, Morykwas MJ. Vacuum-assisted closure: A new method for wound control and treatment: Clinical experience. *Ann Plast Surg* 1997;38:563-577.
- 2) Morykwas MJ, Argenta LC, Shelton-Brown EI, et al. Vacuum-assisted closure: A new method for wound control and treatment: Animal studies and basic foundation. *Ann Plast Surg* 1997;38:553-562.

S2-02

ANALYSIS OF PATHOPHYSIOLOGY OF PRESSURE ULCER IN A MICROCIRCULATORY MODEL

S Ichioka, S Tsuji, N Sekiya, and T Nakatsuka

Department of Plastic and Reconstructive Surgery, Saitama Medical School, Saitama, Japan

Aim: This study aims to establish a pressure ulcers model that visualizes the microcirculation, and to examine the participation of ischemia-reperfusion injury in the pathophysiology of pressure ulcers.

Methods: An original system composed of a new skinfold chamber and compression device allowed loading quantitative vertical stress to the skin. An intravital microscopic technique enabled direct visualization of the microcirculation in the physiological condition and in response to pressure application. To estimate the effect of ischemia-reperfusion injury, animals were divided into two groups: the compression-release group in which the animals received four cycles of compression-release which consisted of 2 hours of compression followed by 1 hour of pressure release; and the compression alone group in which the animals underwent continuous compression for 8 hours. Functional capillary density was quantified before the compression procedure and on day1 (35 hours) after the first evaluation.

Results and Conclusions: The cyclic compression-release procedure significantly decreased functional capillary density as compared to continuous compression, indicating that in our experimental setting repetition of ischemia-reperfusion cycle more severely damaged the microcirculation than single prolonged ischemic insult. The finding supports the significant contribution of ischemia-reperfusion injury to the pathophysiology of pressure ulcers at the level of dynamic in vivo microcirculation.

S2-03

HOW TO TREAT ACUTE PHASE PRESSURE ULCERS WITH NECROTIC TISSUE

Kunio Tsukada, Keiko Tokunaga, and Midori Nagano

Takaoka Ekinan Clinic, Miyagi University, Chiba University

Aim: Most acute phase pressure ulcers have necrotic tissue. Choices to treat such ulcers are controversial. The aim of this study is to help produce a guideline to treat acute phase pressure ulcers with necrosis.

Methods: Records of patients who acute phase ulcers with necrosis were retrospectively reviewed and their primary treatments and outcomes were studied. Ninety-seven ulcers of 86 patients for 3.5 year period were included. Pictures of all ulcers were taken throughout the treatment periods.

Results: Thirteen Stage II ulcers with black or yellow necrosis were treated with hydrocolloid dressings. All of them had excellent results. Forty of 76 Stage III ulcers had yellow necrosis. Hydrocolloid dressings were selected for 24 of them. Two of them became worth. Silver sulfadiazine cream was used for 5 ulcers and the results were relatively good. Fifteen of 36 ulcers with black necrosis were treated with hydrocolloid dressings. Two of them became worth. Thirteen ulcers with black necrosis were resected surgically at the beginnings of the treatments. One of them bled and became worth.

Only one of eight Stage IV ulcers had yellow necrosis and it was treated with hydrocolloid dressing and result was good. Remaining five ulcers with black necrosis were resected with good results.

Conclusions: The primary choice for treating Stage II pressure ulcers with any type of necrosis is hydrocolloid dressings. We recommend hydrocolloid dressings for Stage III and IV ulcers without local symptoms of infection. Then after providing autolysis, surgical debridement should be done. If the patient is malnourished or is under poor pressure release, silver sulfadiazine cream may be recommended.

S2-04

OXYGEN HYPERBARIC PRESSURE THERAPY OHPT FOR DECUBITUS ULCERS

Fukai Takao¹, Nakata Masayuki², and Takeda Akira³

Department of Plastic and Reconstructive Surgery, Saitamakenou Hospital Japan¹, Department of Internal Medicine Saitamakenou Hospital, Japan², Department of Plastic and Reconstructive Surgery, Yokohama City Kowan Hospital, Japan³

Aim: Although oxygen hyperbaric pressure therapy (OHPT) for intractable ulcers is covered by national insurance, few studies have reported its use in the treatment of decubitus ulcers. In this study, we used OHPT as part of conservative treatment, and obtained good results.

Methods: Five inpatients received a 60-min session of 100% oxygen in a Hyperbaric Oxygen Chamber[®] (Kawasaki Engineering Co., Kobe, Japan) at 2 ATA for 10 days. The clinical response was evaluated by four parameters, the difference in the area of ulcer wounds before and after treatment, changes in inflammatory responses, state of wound infection, and histological findings.

Results: OHPT was effective in reducing the area of ulcer wounds in four patients. Three patients, in whom blood tests had shown inflammatory responses (increases in WBC and CRP) before treatment, showed improvement. Two patients, in whom wound cultures were positive for MRSA, became negative after treatment. However, the patients varied in granulation tissue proliferation during treatment. The possible mechanisms underlying these changes were, first, improvement of tissue hypoxia by hyperbaric oxygen supply to ischemic tissue, leading to the above-described reduction in wound area and improvement in inflammatory responses, and second, inhibition of bacterial growth due to an increase in active oxygen species, leading to the disappearance of wound infection.

Conclusions: The results of this study suggest that OHPT is a very useful option in the conservative treatment of decubitus ulcers.

