



Abstracts

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The following compilation of abstracts represents a partial list of submissions received for presentation at the meeting.

GENERAL SESSION I MODERATED BY TOSHIHARU ISHII

NEITHER MYOSIN LIGHT CHAIN KINASE NOR PHOSPHORYLATION OF MYOSIN LIGHT CHAIN INVOLVE THE LYSOPHOSPHATIDIC ACID-DEPENDENT FIBROBLAST-COLLAGEN MATRIX

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Fibroblast-collagen matrix contraction has been widely used as a model system to study the mechanism of human dermal wound healing. The current studies were carried out to know whether myosin II regulatory light chain (MLC) kinase or phosphorylation of MLC involve the LPA stimulated fibroblast-collagen matrix contraction or not. To examine whether or not MLC kinase was important for LPA-stimulated fibroblast-collagen matrix contraction, experiment were carried out using fibroblasts in which MLC kinase expression was knocked down using a siRNA method with or without Rho kinase inhibitor. Further experiments were carried out to test the effects of LPA on MLC phosphorylation with and without Rho kinase inhibitor. MLC phosphorylation by fibroblasts in collagen matrices was assessed by western blotting method using antibodies specific to di-phosphorylated MLC with total MLC as a loading control. Fibroblasts contracted the matrices by LPA even when MLC kinase was knocked down using siRNA, and Rho kinase did not inhibit LPA-stimulated fibroblast-collagen matrix contraction. After LPA stimulation, levels of di-phosphorylated MLC were elevated. Prior addition of Rho kinase inhibitor markedly reduced phosphorylation by fibroblasts under the experimental condition. In conclusion, LPA-dependent gel contraction by fibroblasts does not require myosin II regulatory light chain (MLC) kinase or stimulation of MLC phosphorylation.

HYPERTROPHIC SCARRING IN RAT INCISION MODEL

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Although there are some animal models of wound healing, it has not been fully characterized their scarring. We therefore investigated both 3D-morphological and histological changes of the scarring skin in rat incision models. Under general anesthesia, four full-thickness linear incisions were placed on the dorsum of the each Sprague Dawley rat and Hairless Wister Yagi rat (male, 6 wks old). After re-epithelialization, we took silicone replica from the each incision site until 445 days postwounding. Using these silicon replicas, we quantitatively analyzed the height, volume and the area of the scar by a 3D-profilometer (LIP-50, Science Systems Inc., Japan). The height and the volume of the scar in both rat incision models were significantly increased until 270 days postwounding (350 μ m height, 5 mm³ volume, 270 days postwounding). It showed accumulation of collagen fibers, but not any epithelial hyperplasia in histological analysis. We think that these results showed hypertrophic scarring occurred in these rat incision models. However, there were some different points from the human hypertrophic scarring, e.g., angiogenesis. Therefore, we think that furthermore characterization is needed for the development of the rat incision model as a hypertrophic scarring model.

EXCESSIVE ACCUMULATION OF GLYCOSAMINOGLYCANS IN KELOIDS

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Aim: Keloids are characterized by an excessive accumulation of extracellular matrix ECM. Glycosaminoglycans (GAGs) are one of the major components of ECM in the dermis, which also contains collagens and elastic fibers. Recently, various genes encoding proteoglycan (PG) core proteins have been identified. In this report, we analyzed the GAGs as well as PGs in the keloid lesions.

Methods: Total RNAs were isolated from tissue specimens. The expression levels of various PG core proteins studied by RT-PCR and Northern blot analysis. Immunohistochemical stainings for versican and hyaluronan, and Elastica van Gieson staining were performed.

Results: The expression levels of various PG core proteins, including versican, biglycan, lumican, mimecan and aggrecan increased in the keloid, compared to normal skins. Overexpression of versican was confirmed by immunohistochemical staining. Although both of hyaluronan and elastic fibers are known to interact with versican, only hyaluronan was accumulated in the keloid lesions. Elastic fibers were not detected in keloids by Elastica van Gieson staining.

Conclusions: Our results demonstrated that there was an abnormality in the various components of ECM in keloids. Recently, versican has been reported to play important roles in cell proliferation, migration, and elastic fibers formation. We proposed that an increased accumulation of versican may be involved in one of the key molecules in keloid formation.

WOUND MODEL HEALING USING HR-1 TYPE HAIRLESS MICE: POSITIVE EFFECT OF FOOD WRAP FILM ON HEALING OF TOTAL SKIN DEFECT

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Aim: Closed dressing method using the food wrap film (abbreviated wrap therapy¹) has clinically been applied to a conservative treatment of human pressure ulcers. In the present study, effect of the wrap therapy was examined in the experimental animal model using the back skin of HR-1 hairless mice². This model provides a condition resembling the human skin. Hair removal is unnecessary and the mice possess normal immune functions.

Results: In the experiment, the wrap therapy was proven as effective as the conventional treatments such as administration of basic fibroblast growth factor and prostaglandin-E1.

Considerations: The maintenance of moist environment by wrapping may accelerate the production of endogenous cytokines and hence epithelialization.

Conclusions: The wrap therapy should be cost-saving and convenient choice for treating skin ulcers.

References:

1 Syunichi Toriyabe, Shuzo Suemaru: The wrap therapy was applicable to treating advanced ulcers (grade 3 or 4 classification). Japan Medical magazine 123: 1605–1611, 2000.

2 Jun Ukai, Youko Yoshimura: Effect of including Chinese medicine ointment was examined in the experimental animal model using the back skin of HR-1 hairless mice. Fujita Univ. Medical Association magazine 21(2): 371–390, 2002.

GENERAL SESSION II MODERATED BY YOSHIHIDE OTANI

ABSTRACT

BILIARY STRICTURES AFTER LIVING DONOR LIVER TRANSPLANTATION

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Background: Biliary complications, biliary stricture (BS) in particular, continues to be a significant cause of morbidity after living donor liver transplantation (LDLT). However, the frequency of BS and the outcome of its treatment remain unknown.

Methods: Between October 1996 and January 2004, a total of 135 LDLTs for either adult (n = 114) or pediatric (n = 21) patients were performed at our institution. The grafts were consisted of lateral segment grafts (n = 11), left-lobe grafts (n = 100) and right-lobe grafts (n = 24). The duct-to-duct (DD) biliary reconstruction was performed for 67 cases while the conventional Roux-en-Y hepaticojejunostomy (HJ) was utilized for the remaining 68 cases. The cumulative incidence of BS was compared between the two groups. Furthermore, the management and outcome of its treatment were evaluated.

Results: Overall, BS developed in 31/135 (25.9%) of the cases (DD, 22.0%; HJ, 23.3%). One- and two-year cumulative incidence of BS was 34.5% and 40.6%, respectively in the DD group, while 13.0% and 22.0% in the HJ group (p = 0.03). The graft type was not associated with the incidence of BS. Neither the method of anastomosis (continuous vs. interrupted) nor the use of anastomotic stent was associated with the incidence of BS. The incidence of biliary anastomotic leak was significantly lower in DD as compared to HJ. Biliary sepsis due to BS developed in five cases, all associated with HJ (p < 0.02). Radiological intervention via either percutaneous or endoscopic approach was successful in the majority of the cases, although the recurrence could occur in some. Surgical revision was performed for two cases, both resulted in recurrent stricture.

Conclusions: BS remains frequent complication after LDLT in spite of the technical refinement. The DD anastomosis was associated with the early and high incidence in BS after LDLT. Interventional radiology was a satisfactory treatment of choice and should be the first-line treatment for biliary anastomotic stricture.

ANALYSIS OF PORTAL PRESSURE AND TYPE-1 PLASMINOGEN ACTIVATOR IN THE EXCESSIVE HEPATECTOMY

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Aim: In hepatectomy and liver transplantation, it had been reported that post-operative excessive portal hypertension is a cause of hepatic failure. The purpose of this study is to examine the correlation with portal pressure and PAI-1 which plays an important role in the priming phase of the liver proliferation by using hepatic failure rat model.

Methods: In our findings, the 1-week survival rates of rats in 90% hepatectomy group were all 100%, but all rats in the 95% hepatectomy group died within 96 h. Therefore, we used 90% group as an acceptable hepatectomy model and 95% group as a hepatic failure model. Portal pressures were measured by a transducer after a 27 G needle had been inserted directly into the portal vein. Hepatic gene expression of PAI-1 was examined by real time PCR.

Results: The portal pressure of 95% group was significantly higher than that of 90% group. PAI-1 mRNA levels were significantly increased in 95% group compared with those in 90% group on 5 hr after hepatectomy. Moreover portal pressures were intercorrelate with PAI-1 mRNA levels.

Conclusions: It is suggested that overexpression of PAI-1 which was induced by excessive portal hypertension, is one of the reason of liver failure after massive hepatectomy.

THE INFLUENCE ON MEDICAL ECONOMICS OF THE DRESSING PERIOD FOR WOUND CARE

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Background: Incisional wound dressings are not generally required after the second postoperative day, since epithelial closure of the skin incision is complete in 1 to 2 days in normal skin. In our hospital the wound dressings are usually used for 7 days.

Purpose: In order to change the policy of the dressing period from 7 days to 2 days after surgery, we evaluated the rate of surgical site infection (SSI) and the medical expenditure associated with the dressing materials and antiseptic agents.

Patients and Methods: In a single ward of the Nippon Medical School Hospital, 67 patients underwent surgery in May and October, 2003. The policy of wound care after the closed wound was that the dressing should be changed every day with painting of antiseptic agents for 7 days during the period. In October, the dressings were removed after 2 days except in some situations, such as the incisions with drains or exudative wounds.

Results: It took 3 months to implement the new dressing policy from July to September, 2003. Twenty-six patients in May and 41 patients in October were evaluated.

The consumption of sterile gauze per patient decreased from 242.3 pieces to 184.4 pieces. Iodophors (10% povidone-iodine) also decreased from 275 ml to 225 ml. Hand washes with benzalconium chloride (Welpas) decreased from 0.3 bottle to 0.2 bottle. The rate of SSI decreased from 12% in May to 8% in October. The medical expenditure for wound care decreased by almost \ 1,000 per patient. Film dressings occasionally caused perspiration or dermatitis.

Conclusion: The policy of the 2-days dressing for wound care did not increase SSI and decreased the consumption of sterile gauze and antiseptic agents.

GENERAL SESSION III MODERATED BY AKIO YAMAGUCHI

THE RESULT OF SSI SURVEILLANCE IN THE DEPARTMENT OF SURGERY, TSUSHIMA CITY HOSPITAL

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Background and Aim: The surveillance of SSI (surgical site infection) was started from the April of 2003 in our department. The difference concerning SSI between 2002 and 2003 was clarified.

Patients and Methods: All surgical services of our department, 499 operations including 92 emergencies in the year of 2002 and 457 including 91 in 2003 (P = 0.692) were enrolled in this study. No statistical difference in age and gender was recognized between the two groups. The incidence of SSI and the infected bacterium were surveyed in each group.

Results: The rates of SSI in all surgical operations were 14.8% in 2002 and 10.5% in 2003 (P = 0.283), in the elective operations 11.1% and 8.5% (P = 0.470), and in the emergent operations 31.5% and 18.7% (P = 0.035), respectively. The enterobacteria were identified in 16 cases among 29 infected wound in 2002, and 18 among 23 in 2003, respectively. MRSA and/or MRSE were identified in 6 cases in 2002, 2 in 2003, respectively.

Conclusion: The rate of SSI was improved from 14.8% of 2002 to 10.5% of 2003. The decrease was the result of three interrelated factors: the regular reports of the SSI surveillance to surgical staffs, prophylactic administration of antibiotics during operation and the change of hand gloves of operative staffs after the contaminated manipulations during operations.

RETROSPECTIVE ANALYSIS OF THE OPERATED CASES OF GASTRO-DUODENAL ULCER PERFORATION

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Aim: We introduced the laparoscopic omental implantation from 1992. In the early stage, stenosis was listed in the exclusion criteria of it, and *Candida* infection was not recognized as an important factor. The operated cases of gastro-duodenal ulcer perforation were analysed retrospectively. Indication of the laparoscopic omental implantation and clinical significance of the *Candida* infection were discussed.

Patients: From 1993 to September 2004, 58 patients undergone surgery for stomach or duodenal perforation at the Keio University Hospital were enrolled in the present study.

Result: *Candida* was positive in 8 cases (40%) out of 20 cases performed culture of ascites taken within 24 hours after onset of the abdominal pain. Eight cases had wound infection and *Candida* was detected in six cases. Intra-abdominal abscess was found in one case and *Candida* was detected in this patient. In a specimen of the resected duodenum, *Candida* colonized at ulcer base with massive infiltration of granulocytes surrounded the hyphae of *Candida*. We presented the cases with duodenal stenosis detected preoperative endoscopy. None of them showed symptom of the stenosis after the laparoscopic omental implantation.

Conclusion: It was suggested that *Candida* infection is important as postoperative complications. Furthermore, it was speculated that *Candida* is involved in the process of the gastrointestinal perforation. If the patient could eat foods before the onset of abdominal pain, the stenosis detected by preoperative endoscopy is not enough to exclude the application of laparoscopic omental implantation.

GENERAL SESSION IV

MODERATED BY YOSHIMITSU KUROYANAGI

TREATMENT OF INTRACTABLE ULCERS USING ARTIFICIAL DERMIS AND BASIC FIBROBLAST GROWTH FACTOR-SCANNING ELECTRON MICROSCOPIC OBSERVATION OF MOLDED BLOOD VESSELS

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This study involves the application of a combination method that employs an artificial dermis, the silicon seat of which has a defect that is 3 mm in diameter, and 1 µg bFGF for full-thickness skin defects (1 × 1 cm) to the bare cartilage in both ears of 18 rabbits. The wounds that received the combination therapy were divided into three groups, which were administered bFGF 3, 5, and 7 days after wound preparation. As a control, skin deficit wounds with only an artificial dermis were made. After 14 days, tissue samples were observed.

In all the groups, combination therapy wounds showed good granulation. In the 3-day group, it was observed that combination therapy wounds had a higher degree of vascularization from the wound margins than the control wounds. In the 5- and 7-day groups, combination therapy wounds showed a high degree of vascularization from their margins and a blood plexus at the center of the wound, and the perichondrium completely covered the floor of the wound. In each of the groups, no differences in the expression of anti-bFGF antibody were observed in either wound.

It was concluded that combination therapy using an artificial dermis and bFGF can accelerate tissue regeneration in intractable ulcers ranging from those with full-thickness skin defect to those with a bare cartilage.

SIMULTANEOUS GRAFTING OF ARTIFICIAL DERMIS AND SPLIT THICKNESS SKIN GRAFT

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Aim: We have investigated the effects of PDWHF (platelet-derived wound healing factor), cultured endothelial cells and fibroblasts on wound angiogenesis in cases of the artificial dermis (AD) grafting in rat experimental model¹. When the AD was treated with the PDWHF and the cultured cells, simultaneous grafting of the AD and the split-skin was possible. On the basis of this result, the clinical trial was performed.

Methods: In a case of 49% TBSA burn, full-thickness burned wound on his leg was treated by this method. The artificial dermis was treated with autogeneous PDWHF together with allogenic cryopreserved cultured endothelial cells and fibroblasts.

Results: The skin graft was completely taken and satisfactory tissue texture was obtained. Histological examination revealed that bovine collagen tissue lasted in the dermis for 1 month after surgery.

Conclusions: The present study revealed that treatment with PDWHF, combined with cultured endothelial cells and fibroblasts accelerated wound angiogenesis. By this method, one-step grafting procedure of the AD and the split-skin is possible.

Reference:

1. Soejima K, Xin Chen, Nozaki M, et al: Effects of platelet derived wound healing factor (PDWHF) in cases of artificial dermis grafts –experimental study in rats. J. Jpn. P.R.S. 23:291–299, 2003.

CULTURED SKIN WITHOUT FIBROBLASTS

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Aim: We have developed cultured skin using two kinds of collagen sponges. Keratinocytes can be cultured without fetal bovine serum but fibroblasts cannot be cultured without serum. In this study, we tried to produce the cultured skin without fibroblasts and graft it.

Materials and Methods: Dermal collagen sponge (DCS) with a pore size of 90 µm and epidermal collagen sponge (ECS) with a pore size of 15 µm were prepared. Human keratinocytes were seeded onto an ECS with a seeding density of 100,000 cells/cm² and the seeded ECS was put on a DCS in which basic fibroblast growth factor (bFGF) was incorporated instead of fibroblasts. The cultured skin without fibroblasts was grafted onto full-thickness skin defects on the back of severe combined immunodeficient mice. The mice were divided into three groups; (1) single application of bFGF with a dose of 20 µg/cm², (2) sustained release of bFGF with a dose of 1 µg/cm², (3) sustained release of bFGF with a dose of 20 µg/cm². Two weeks after grafting, the specimen was harvested and histological observation was performed.

Result: Epithelialization was completed only in group (3). Basement membrane was also formed immunohistochemically.

Conclusion: Cultured skin without fibroblasts but with incorporation of sustained release of bFGF with an initial dose of 20 µg/cm² took successfully.

IMPORTANCE OF THE PERIOSTEUM FOR THE RECOVERY FROM INJURY: APPLICATION OF ARTIFICIAL DERMIS TO FULL-THICKNESS SKIN-DEFICIENT INJURY WITH BONE EXPOSURE

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Aim: The recovery from injury always requires development of blood vessels and fibroblasts. Injuries impairing blood circulation, diseases such as diabetes, and aging delay the recovery from injury. We produced full-thickness skin-deficient injury with bone exposure as a model of injury with poor blood circulation, and evaluated the recovery under various conditions using the degree of neovascularization.

Methods: A full-thickness skin-deficient injury, measuring 15 mm × 10 mm, was made on the parietal region of normal Wistar rats, diabetes model rats, and aged rats. These animals were classified into the periosteum removal group (exposure of the cranial bone) and the periosteum retaining group, and artificial dermis was applied to the injury. Growth of granulation tissues was observed, and the density of neogenetic blood vessels growing into the injury was quantified.

Results: The density of blood vessels in the normal parietal tissues in the normal group was 80/mm². In the periosteum retaining group, the density of blood vessels was recovered to the same level as that in the normal group about 14 days after surgery in the diabetes and aged groups. In the periosteum removal group, the recovery of the density of blood vessels required more than 20 days in the normal group and about 1 month in the diabetes and aged groups.

Conclusions: The development of fibroblasts, extension of blood vessels, displacement by collagen fibers, and achievement of epithelialization were significantly delayed in the periosteum removal group compared to the periosteum retaining group. These findings suggested the importance of the periosteum for the recovery from injury.

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1. Naoto Yamada, Nobuyuki Shioya, and Yoshimitsu Kuroyanagi. Evaluation of an allogeneic cultured dermal substitute composed of fibroblasts within a spongy collagen matrix as a wound dressing. *Scand J Reconstr Hand Surg* 1995; 29: 211–219
2. Annie F. Black, Francois Berthod, Nicolas L'heureux, et al. In vitro reconstruction of human capillary-like network in a tissue-engineered skin equivalent. *The FASEB Journal* 1998; vol.12 October: 1331–1340

WOUND REPAIR FOR FAT GRAFTING

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Aim: Recent advances in regenerative medicine have opened up the options of materials used for transplantation. However, only a few studies have examined the take of transplanted tissues. We tried to establish a functional bed for transplanted tissues using growth factors.

Methods: A cylinder-type silicone substrate (spacer) was coated with the photoreactive gelatin containing growth factors (VEGF and bFGF). This spacer was transplanted into the dorsal subdermal layer in a rabbit. After 2, 3, and 4 weeks, the capsule formed around the spacer was histologically assessed for use as a transplant bed. In addition, after 2 to 4 weeks of spacer grafting, autologous fat was transplanted into the capsule. After 4 more weeks, the fat grafted was assessed immunohistochemically to evaluate the capsule as a functional bed for transplantation.

Results: In the groups pretreated with growth factors, proliferation of blood vessels was observed in the capsules. After fat grafting, the patterns of overall necrosis were observed in controls. However, good proliferation of blood vessels and favorable fat taking were observed in the groups pretreated with growth factors. Necrosis, however, was found at the center of the grafted fat.

Conclusions: A vascularized transplant bed was useful for promoting take of the grafted fat.

GENERAL SESSION V MODERATED BY YASUNORI OKADA

THE EXPRESSION PATTERN AND THE FUNCTION OF A NOVEL PROTEIN AGK114 DURING WOUND HEALING

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Aim: A novel protein AgK114 is expressed restrictedly on dermal sheath cells near sebaceous glands in normal skin, however, its expression is strongly and transiently induced after selected skin impaired stimuli [1]. These findings suggest that AgK114 is involved in the wound healing response, but this remains undemonstrated. In this study, we report the effects of this novel protein on wound healing.

Methods: Full-thickness excisional wounds were made on the depilated dorsal skin of CD-1 mice and mAgK114FL protein was administered onto the wounds. Wounded skins were collected and immunohistochemical study was performed. Wound closer was determined at days 4 and 7. In addition, the amounts of wound response proteins in tissue homogenates prepared from impaired skin were measured by EIA.

Results: Expression of endogenous mAgK114 was induced in the initial 24 h at the edge keratinocytes and enhanced strongly during keratinocyte migration, followed by disappearance once epithelialization was complete. mAgK114FL protein promoted wound closure and the formation of blood vessels in granulation tissue. mAgK114FL also up-regulates proMMP-9, vascular endothelial growth factor, transforming growth factor- β , IL-6, and IL-1 β production during the early stage of wound healing.

Conclusion: The findings suggest that mAgK114 mediates the wound response during the healing process, and promotes wound repair.

Reference:

- [1] Tatefuji T et al. Identification of the novel membrane-associated protein AgK114 on hamster keratinocytes recognized by a monoclonal antibody K114. *Biol. Pharm. Bull.* 2004; 27, 1742–174

THE ANALYSIS OF THE ROLE OF CYCLOPAMINE IN ADULT CUTANEOUS WOUND HEALING

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Aim: We previously reported the expression pattern of sonic hedgehog (shh) in adult and fetal wound healing in mice. This time, we intradermally injected cyclopamine, the chemicals that block signaling of shh, to know the role of shh during cutaneous wound healing.

Methods: 8–12 weeks male ICR mice were intradermally injected with various concentration of cyclopamine. Immediately after injection, full-thickness cutaneous wounds were made. Cyclopamine was injected every 24 hours for 7 days. Eight days after, the animals were killed and the samples were examined histologically.

Results: The thickness of epidermis at the margin of the wound was significantly thicker in the samples treated with cyclopamine dose dependently.

Conclusions: Shh is transiently expressed at the margin of the epidermis where the thickness of epidermis is thickest. By blocking of shh, the treated epidermis became thicker. There may be some mechanism of negative feedback in the shh signaling in adult wound healing.

PATHOPHYSIOLOGICAL ROLES OF IFN- γ IN SKIN WOUND HEALING

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Aim: We examined the pathophysiological role of IFN- γ in the skin wound healing process utilizing wild-type (WT) and IFN- γ KO mice.

Materials and methods: Excisional skin wounds were made on the back of WT and KO mice. Thereafter, we evaluated macroscopic wound closure, leukocyte infiltration, collagen accumulation, and angiogenesis. Moreover, Western blotting analysis was performed on Stat1, Smad2, and Smad7.

Results: In comparison with WT mice, KO mice exhibited an accelerated wound healing as evidenced by rapid wound closure and enhanced granulation tissue formation and angiogenesis. IFN- γ KO mice also exhibited. Furthermore, the absence of IFN- γ augmented the TGF- γ 1-mediated signaling pathway, as evidenced by increases in the levels of total and phosphorylated Smad2 and a reciprocal decrease in the levels of Smad7.

Conclusion: These results demonstrate that there is crosstalk between the IFN- γ /Stat1 and TGF- β 1/Smad signaling pathways in the wound healing process.

GENERAL SESSION V

BONE FORMATION IN POROUS HYDROXYAPATITE BLOCK INDUCED BY ATTACHMENT OF RIB-LATISSIMUS DORSI PERIOSTO-MUSCLE FLAPS IN RABBITS

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Introduction: The present paper describes the sequential event of induced bone formation in vivo using porous hydroxyapatite (PHA) with an attachment of vascularized periosteal flaps.

Materials and methods: PHA blocks (porosity; 50%, dimensions; 8 × 4 × 2 mm) were ligated to the cambium layer of periosteum and muscle followed by raising of latissimus dorsi musculoperiosteal flap in rabbit. PHA blocks were removed 4, 8, and 12 weeks after the operation, and these blocks were performed histological examination as well as three-dimensional reconstruction of induced tissue in the pores using serial section of the specimen. Furthermore, to go insight into bone induction, an extent of that was discussed, and correlations between a calculation of ossification ratio indicated as "area of calcified bone/area of the pore" in each pore and distance from attached periosteum were also discussed.

Results: Fibrous tissue containing capillary filling each pore is encompassed with sloughing bone that connects through the interporous canal each other. Fat having erythroblast islets is also demonstrated in some pores. Adipocytes were found surrounding capillary near the center of the pores. Furthermore, there was a distinct negative linear correlation between bone formation and distance from attaching periosteum. On the other hand, no bone and fat formation were found in the pores with an attachment of muscle.

Conclusions: An induced bone formation was usually accompanied by an appearing of fat containing hematopoietic cells and capillary in vivo, and this event starts from the pores closer to the periosteum.

BASIC FIBROBLAST GROWTH FACTOR STIMULATES HUMAN KERATINOCYTE MOTILITY BY RAC ACTIVATION

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Topical application of human recombinant basic fibroblast growth factor (bFGF) promotes wound healing including reepithelization. bFGF, however, has been reported to have little in vitro effects on keratinocyte compared to other cell types such as endothelial cells or fibroblasts. The aim of this study is to investigate the mechanisms of bFGF-stimulated keratinocyte migration. Normal human keratinocytes, seeded on coverslips which were noncoated or coated with type I collagen or fibronectin, were treated with or without 10% FBS, bFGF and KGF to examine their ability to spread and make focal adhesion. Morphologically, there were no significant changes among in the presence or absence of any growth factors, on the experiments using non- or fibronectin coated coverslips. Keratinocytes formed lamellipodia only when they were stimulated with bFGF on the collagen-coated coverslips. Next, we evaluated the effects of bFGF and KGF on keratinocyte migration by Boyden chamber assay. Keratinocyte migration was significantly enhanced not only by KGF but also bFGF. These results induced the hypothesis which bFGF could activate Rac, a member of Rho GTPase, when keratinocyte attached on type I collagen. So further experiments were carried out to establish the hypothesis. We employed pull-down assay to detect GTP-loaded Rac (an activated form). GTP-loaded Rac was detected only in the lysate of bFGF-stimulated keratinocytes on collagen-coated dishes not on non-coated dishes. This in vitro study first demonstrated that bFGF exerts stimulatory effect on keratinocyte migration under the presence of type I collagen as a scaffold, and, at least, Rac activation is involved.

GENERAL SESSION VI

MODERATED BY HIROHARU ISOMOTO

TWO CASES OF A REFRACTORY WOUND FOLLOWING SURGERY FOR PERITONITIS SUCCESSFULLY TREATED WITH A BFGF PREPARATION

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[Introduction] We experienced two cases of a postoperative wound successfully treated with a bFGF preparation, for which creation of an artificial anus at the wound site could not have been avoided following surgery for peritonitis due to colonic perforation. The details are reported herein.

[Case 1] The patient, a 66-year-old man, was admitted with a chief complaint of constipation. At complete examination, a diagnosis of sigmoid colon obstruction due to a recurrence of colon cancer was established.

[Clinical Course] With a concurrent disease of general peritonitis, the patient underwent an emergency laparotomic drainage procedure. As an elevation of the intestine was deemed difficult because of cancerous infiltration, a loop stoma was created at the wound site with the cecum having been lifted. After this surgery, albeit a wound infection caused by fecal contamination was noted, as a result of facilitating a procedure to protect the wound from fecal contamination, washing the wound and administering the bFGF preparation, a satisfactory granule formation was observed at the sixth postoperative week.

[Case 2] The patient, a 73-year-old woman, was admitted with a chief complaint of abdominal pain. A CT scan revealed an intraperitoneal abscess and she was diagnosed with peritonitis.

[Clinical Course] The patient underwent an emergency laparotomy procedure. An abscess was located within the mesentery associated with perforated diverticulum of the transverse colon. The contaminated intestine was resected and due to severe inflammatory adhesion, the ascending colon was opened at the wound site, followed by creation of a single stoma. After this procedure, the patient experienced a comorbidity of a wound infection but at the 10th postoperative week, healing of the wound was attained by virtue of washing the wound and administering the bFGF preparation.

[Conclusion] After surgery for peritonitis, although the postoperative wounds, at which sites the creation of a stoma could not have been avoided, were of a refractory nature, improvement in the postoperative wounds was noted in a short space of time through the application of bFGF preparation.

NURSING CARE FOR EARLY COMPLETION OF PEG STOMA: REPORT OF 13 CASES

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Aim: To clarify the factors that induce peristomal wound infections after percutaneous endoscopic gastrostomy (PEG) replacement. And to design the nursing cares for prevention of the infection.

Patients and Methods: Thirteen cases including 8 males and 5 females were enrolled in this study. The mean age was 74.8 years old. The diseases of these cases included 10 cerebrovascular diseases, one brain contusion, one Alzheimer's disease, one chronic respiratory failure. Six cases were affected with aspiration pneumonia. The changes of appearance around PEG stoma were assessed retrospectively depending on the medical records of these cases.

Results: Peristomal wound infection was identified in 8 cases. On 5 cases among them, the infection healed within 7 days after the PEG replacement. The remnant 3 cases showed prolonged and severe wound infection. It took 2 to 3 weeks to heal the infections.

Conclusion: Peristomal infections might be induced by both inappropriate oral sterilization before PEG replacement and the retention of secrete around the stoma. To prevent peristomal infection, the sterilization of both oral cavity and abdominal wall before PEG replacement and daily meticulous care for stoma are essential.

THE REPORT OF ONE ESOPHAGEAL CANCER CASE: MICROSURGICAL LYMPHATICOVENOUS IMPLANTATION AND ANASTOMOSIS FOR AXILLARY LYMPHORRHEA

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The case was 56-year-old man (esophageal cancer, multifocal, T3N4M0). He was performed subtotal esophagectomy with three-field lymph node dissection under thoracalaparotomy, and reconstructed by gastric tube through posterior mediastinal route. One month after operation, left axillary lymph node dissection was performed and irradiated (60Gy). After these treatments, he had axillary lymphorrhea for a long time (300 ml/day) and the wound did not close. Though the other wound were healed and the ingestion was possible, he could not leave the hospital only for this problem. So we performed one microlymphaticovenous anastomosis at the left arm and two microsurgical lymphaticovenous implantations at the left wrist and elbow. Just after this operation, the lymphorrhea decreased remarkably and wound healing was completed. And he could leave the hospital 3 weeks after this operation. In this case, the cause of lymphorrhea and the mechanism of wound healing after lymphaticovenous anastomosis were explained clearly by anatomical point of view. And the effect of this technique was clear in this case. So we presented it as one valuable case by the reason that mentioned above.

GENERAL SESSION VII MODERATED BY NORIO KUMAGAI

EFFICACY OF PLATELET-RICH PLASMA BASED FIBRIN GLUE IN THE CUTANEOUS SUTURE WOUND AND GRAFT DONOR SITE

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Aim: Platelet-Rich Plasma based fibrin glue (PRP glue) contains a variety of growth factors, and the synergistic effect amongst these factors contributes to the enhanced wound healing and reduced scar formation in addition to the known anti-inflammatory properties of fibrin glue. The purpose of this study was to evaluate the effect of PRP glue on cutaneous wound healing and scar formation.

Method: Three split-thickness skin graft donor sites and four hypertrophic scar revision wounds were included in this study. PRP was made from 40 ml of autologous whole blood and was mixed with commercially available fibrin glue. Each wound surface was divided into two halves. PRP glue was applied to one half, while the contralateral half was treated with only fibrin glue as control. All wounds were dressed with film dressing and left covered for a week.

Results: There were no discernable differences in the level of epithelialization of the graft donor sites. However, one week postoperatively, redness of the wound surfaces was less on the PRP glue site compared with the control site. Dermatoscopic examination showed congestion of the surface vessels was greater on the control halves compared with the PRP glue halves. Six months postoperatively, scar suppression was observed in one graft donor site, and three out of the four scar revision cases showed suppression of hypertrophic scar formation.

Conclusion: This study shows that application of PRP glue could potentially affect neovascularization of the wound surface and suppress scarring in cutaneous wounds, as a result of growth factor networks in PRP glue.

THE TREATMENT OF FACIAL ANGIOFIBROMAS OF TUBEROUS SCLEROSIS USING AUTOLOGOUS CULTURED EPITHELIUM

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Tuberous sclerosis is a syndrome of autosomal dominant inheritance that has predominant symptoms of facial angiofibromas, convulsive seizure and intelligence impairment. The facial angiofibromas are usually treated with laser resurfacing or dermal abrasion. However these treatments cannot be enough to shave to the deep dermal layer considering the risk of hypertrophic scar in Asian. This is why recurrences occur in short term and we need to treat frequently. We grafted autologous cultured epithelium on the wound shaved to the deep dermal layer and obtained satisfactory result for rather long term. We shaved epidermis with grinder and then shaved remaining angiofibromas with razor as smooth as possible. We grafted autologous cultured epithelium on it. From 1994 to 2003, five patients, three were males and two were females; were treated using this method and obtained satisfactory result. There was no hypertrophic scar and no recurrence for 9 years at most. Histopathological findings of facial angiofibromas are the increasing of collagen fiber and capillary vessels from dermal papillary layer to deep dermal layer. These findings suggest that shaving to the superficial layer remains most part of the angiofibromas and it occurs recurrence at the early stage. Using autologous cultured epithelium makes it possible to shave to the deep dermal layer and obtain satisfactory result.

THE USEFULNESS OF SPLIT-THICKNESS SKIN GRAFT FOR CLEANING AN INTRACTABLE STERNAL WOUND INFECTION

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Aim: We report the usefulness of split-thickness skin graft as one of the treatments for cleaning an intractable sternal wound infection.

Patient and Method: A 73-year-old male with thoracic aorta aneurysm treated with sternotomy had a sternal wound infection after the operation. Debridement and omentum flap coverage had been performed, but most of the omentum flap had dropped out. Our division was requested for the treatment of the wound because control of infection is difficult, even if performing wound washing. Vacuum-Assisted Closure (VAC) treatment, etc. The wound was wide open from the episternum to the xiphoid process, and puriform discharge was seen not only from the sternum bone marrow, but also from the surrounding soft-tissue. Because deep infection was suspected, we judged that the coverage of open wound by muscle flap was not adequate at the time, and we decided to perform meshed split-thickness skin graft for cleaning the wound bed and focusing the infected area.

The skin graft took on the noninfected area, and the raw surface was reduced by about 1/4, by doing debridement and split-thickness skin graft two times. Lastly, we covered the open wound perfectly by rectus abdominis muscle flap.

Conclusion: The split-thickness skin graft contains cytokines and growth factors related to the regeneration of the skin and has the potential ability to clean an infected wound. Therefore the split-thickness skin graft can accelerate the cleaning of the infected wound by epithelialization and this is one of useful treatments for an intractable sternal wound infection.

TREATMENT FOR MRSA INFECTED STERNAL OSTEOMYELITIS USING VACUUM-ASSISTED CLOSURE WITH IODINE CADEXOMER OINTMENT

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Sternal osteomyelitis with MRSA infection after cardiovascular surgery presents is a difficult treatment challenge. In this series of patients, cadexomer ointment containing iodine and then administered vacuum assisted closure therapy using hydrophilic polyurethane dressing material and silicone tube for drainage.

This therapy was used in five cases. One of them is a case in which after reconstruction with rectus abdominis myocutaneous flap following debridement for sternal osteomyelitis, MRSA infection developed and the wound opened. The wound was closed by this therapy. In the remaining four cases, MRSA became negative. In one case of them, epithelialization could be obtained by this therapy. In three other cases, debridement and reconstruction with rectus abdominis myocutaneous flap were performed because a fistula remained.

Important advantages of vacuum-assisted closure are removal of excessive edema fluid with increase in tissue blood flow, associated decrease in the amount of bacteria, and movement and growth of tissue surrounding the wound in response to the mechanical force of suction pressure. However, in MRSA infected sternal osteomyelitis, we sometimes find it difficult to treat it with the vacuum-assisted closure alone. Considering that combining the vacuum-assisted closure with cadexomer ointment containing iodine may be effective for suppressing MRSA infection. Cadexomer is a beads-shaped dextrin polymer, and it cannot be absorbed by hydrophilic polyurethane dressing material in the vacuum-assisted closure, but remains and has the water absorbing activity, so it is considered effective for removing redundant interstitial fluid.

**GENERAL SESSION VIII
MODERATED BY SHIGEHICO SUZUKI**

TREATMENT OF EXPOSED SYNTHETIC MATERIALS WITH NEGATIVE PRESSURE THERAPY

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Infected wounds with exposed synthetic materials are challenging for surgeons. Excisions and replacements with well-vascularized materials are standard procedures but accompany high morbidities of these procedures. Recently, these wounds could be treated with negative pressure therapy.

We treated with three cases with exposed synthetic materials with negative pressure therapies. Exposed PTFE graft with MRSA infection was treated with negative pressure therapy for 3 weeks. MRSA sepsis developed and NPT was discontinued. Later, excision of infected graft and bypass surgery was performed without complications. Two cases with exposed marlex mesh graft for ventral hernias were successfully treated with combinations of local wound debridement, negative pressure therapies, with skin grafts. The treatment required multiple debridements of tips of exposed mesh and frequent wound irrigations. Six months after starting the negative pressure therapy, no hernia recurrence or wound infection was observed.

The preliminary results obtained with negative pressure therapies are encouraging for high-risk patients with infected synthetic mesh grafts after hernial repair.

USE OF KITCHEN WRAP AS A WOUND DRESSING

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Introduction: Based on the principle of wound healing, conditions for qualities a wound dressing should are that: it must maintain a moist environment for the wound, it must match large and small-shaped wounds, it must not cling to the wound, treatment should be simple, and the cheaper it is, the better. Kitchen wrap is a material that satisfies the above conditions. We have performed wrap therapy for pressure ulcers at this hospital for some time, and we have used this expertise in general wound treatment as reported here.

Wrap therapy: Without exception, the wound was not disinfected and was irrigated with tap water. The wound was then covered with kitchen wrap than had in no way been sterilized.

Case presentation: 1) Massive pressure ulcer, 2) burns on the hands, 3) burns on the feet, 4) frostbite over a vast area extending to both lower legs in their entirety, 5) crushing of a finger caught in machinery, 6) sores on the lip that had not healed after a long period of time, and 7) conjunctivitis that had not healed over a long period of time.

Discussion: Kitchen wrap is extremely applicable and useful in treating vast pressure ulcers, wounds, burns, irregularly shaped wounds, wounds causing pain because of adherence to dressings, and the like. It is a revolutionary dressing that demolishes conventional methods of treatment. Informed consent was obtained from patients with regard to the fact that the wrap used was not for medical use.

WOUND BED PREPARATION (WBP) BY MDT (MAGGOT DEBRIDEMENT THERAPY)

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Background: Patients who suffer severe ischemic limb ischemia (ulcer, necrosis of foot) and diabetic foot (Wager classification 5) has lots of co-morbidity and risk factors (severe diabetes, hypertension, chronic renal failure, coronary arterial diseases, cerebral arterial diseases, neuropathy, and so on). Due to those factors sometimes we should choose major limb amputation instead of applying reconstruction of artery or soft tissue (skin and muscle). We are now introducing Maggot debridement therapy (MDT) for wound bed preparation (WBP) of such ischemic ulcers.

Methods and Results: We experienced 5 MDT cases and so far those results are good.

Conclusion: Those case studies offer an insight into the practicalities involved in the use of maggots as effective and safe debriders of tissue for WBP. MDT is an underused and misunderstood resource when considering effective wound care and should be actively considered as an alternative therapy in wound care. In this meeting we want to present our experiences to the audience and have the discussion to those results.

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GENERAL SESSION IX MODERATED BY KENJI TAZAWA

THERAPEUTIC ANGIOGENESIS FOR ISCHEMIC LIMB BY AUTOLOGOUS TRANSPLANTATION OF BONE-MARROW CELLS

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Autologous transplantation of bone marrow-mononuclear cells was performed in 27 limbs of 26 patients with nonhealing ischemic ulcer ($n = 17/27$), rest pain ($n = 1/27$) and intermittent claudication ($n = 9/27$) due to peripheral arterial occlusive disease. Nine had arteriosclerosis obliterans (ASO), 17 had Buerger's disease and one had vasculitis. We injected with the upper limb in 3 and the lower limb in 12. The ulcer improved in 50%, the rest pain and intermittent claudication improved in 77.8%. In 5 out of 8 limbs, the ulcer exposed of the tendon and the bone had not healed dramatically. Four weeks and 6 months after transplantation, efficacy of this procedure were evaluated by Visual Analog Scale (VAS), the ankle-brachial index (ABI) and Laser Doppler blood flow analysis (LDBF). The VAS improved significantly at 4 weeks ($p < 0.001$) and 6 months ($p < 0.001$) and ABI improved significantly at 4 weeks ($p = 0.026$) and 6 months ($p = 0.009$). The LDBF did not improve significantly at 4 weeks and 6 months.

PROSPECTIVE EVALUATION OF CALF MUSCLE PUMP FUNCTION IN PATIENTS WITH CHRONIC VENOUS INSUFFICIENCY USING NEAR-INFRARED SPECTROSCOPY

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Aim: To compare the calf muscle pump function between patients with early, and those with advanced chronic venous insufficiency (CVI).

Methods: A total of 103 limbs in 90 patients with of primary valvular incompetence were included. The clinical manifestations were categorized according to the CEAP (clinical, etiologic, anatomic, and pathophysiologic) classification, and the patients were divided into two groups: group I ($C_{1-3}E_pA_{S,D,P}P_R$) and group II ($C_{4-6}E_pA_{S,D,P}P_R$). The distribution of venous insufficiency including superficial (SVI), deep (DVI), and perforating vein insufficiency (PVI) was determined using duplex scanning. The HHbFI is the average rate of increase in the venous volume. The HHbEI is the percentage of the volume ejected after one tip toe maneuver. After a new plateau is reached, the patients were asked to do 10 tiptoe movements, and HHbRI is measured.

Results: There were significantly higher values in the HHbFI and HHbRI in group II than group I ($p = 0.0004$ and 0.0006 , respectively). In group II, HHbRI was found to have significantly higher value in patients with DVI than SVI. Similarly, patients with PVI had significantly higher value in HHbRI in group I and II compared to these with SVI.

Conclusions: Patients with advanced CVI may have increased calf muscle pump malfunction. Increased HHbFI and HHbRI could reflect the advanced CVI in patients with DVI and PVI.

EFFICACY OF THERMOGRAPHIC ASSESSMENT FOR HEALING PROCESS OF PRESSURE ULCER: A CASE REPORT

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Objective: Thermographic imaging was reported superior to clinical examination in estimating pressure ulcers. In this study, we investigated the efficacy of thermographic assessment in a patient with large pressure ulcer treated by buried chip skin grafting.

Method: A 54-year-old bedridden male, who suffered progressive general paresis by peripheral nerve disorder, was admitted to Tannan Regional Medical Center with Stage IV pressure ulcer on the sacral. The ulcer was 16.3×14.3 cm in size with massive necrosis. After repeated debridement and wound care, the ulcer was covered with well-vascularized granulation tissue within three months. To accelerate wound closure, we performed buried chip skin grafting. Thermographic imaging, as well as clinical examination, was estimated every one week for eight weeks from just before the operation.

Result: Before skin grafting, granulation tissue was equally generated clinically all over the ulcer, whereas scattered cooler areas were observed in thermographic imaging. One week after skin grafting, no epithelialization from grafted skin was confirmed clinically, some of the grafted points were observed as warmer spots in thermographic imaging. These warmer areas were extended 2 week after skin grafting and small epithelialized areas was confirmed clinically in the center of these warmer areas. These warmer areas observed by thermography were confirmed as epithelialized areas with following clinical examination. Through 8 weeks investigation, observation by thermographic imaging was superior to clinical examination. The ulcer was reduced gradually except some areas in accordance with preoperative cooler areas and postoperative nonwarmer areas. In these areas, nursing care assessment revealed that wound healing was delayed by sustained mechanical friction.

Conclusion: In estimation of a process of healing pressure ulcer, it was suggested that thermographic imaging was superior to clinical examination.

CHANGE OF CYTOKINE IN PRESSURE ULCER WITH UNDERMINING

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The purpose of this study was investigated the change in cytokines in pressure ulcers with undermining and relationship between its changing and wound healing process. We investigated cytokines in pressure ulcers with undermining and wound change patterns in order to clarify cytokine behaviour at each status. Monthly measurements were performed of the levels in retained exudate of the cytokines; IL-1 α , IL-1 β , bFGF, PDGF-AB, IL-4, TGF- β 1, and VEGF. Wounds were observed weekly. All subjects had consented to participate in the study. Cytokine measurements were performed at six wound sites. Only IL-1 α , 1 β and VEGF were found in detectable quantities in all exudate samples; all of the others were undetectable at various times. Pressure ulcers with delayed healing at the time of the study had lower rates of detection of IL-4 and bFGF compared with other pressure ulcers. Cytokines were increased at each wound change: for undermining adhesion, it was five out of seven (elevation in VEGF); for wound contraction, three out of four (bFGF); for increased granulation on the side of the undermining, three out of three (bFGF); and for epithelialization also three out of three (bFGF). Therefore, it was suggested that VEGF and bFGF were related to the promotion of healing in the pressure ulcers with undermining.

A CASE OF REFRACTORY DECUBITUS ULCER SUCCESSFULLY TREATED WITH A BFGF PREPARATION COMBINED WITH A WOUND COVERING AGENT AND NUTRITIONAL CARE BY NST

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[Introduction] At our institution, care for decubitus ulcer is conducted with the cooperation of NST and the Decubitus Ulcer Management Committee. Recently, satisfactory results were produced in a case of this condition by combining nutritional care by NST and the application of a wound covering agent and a bFGF preparation. The details are reported herein.

[Case] The patient, a 75-year-old woman, was admitted with COPD and cerebral anoxia. She developed a consciousness disorder 5 months following admission and became dependent on artificial respiration. Shortly thereafter, she began to suffer from a decubitus ulcer located in the sacral region.

[Evaluation] The macroscopically observable ulcerous condition was sketched and its features described. A DESIGN (to evaluate the clinical course of the decubitus ulcer) was conducted and the condition was quantitatively rated for sequential evaluation.

[Clinical Course] At the initial examination, an ulcer (classified as stage III according to the NPUAP classification) was found at the sacral region. For treatment, the necrotic tissue was removed through débridement and the wound was washed and sterilized. Because no improvement was noted, treatment by NST and the Decubitus Ulcer Management Team was initiated. A gastric fistula was created and feeding was changed from TPN to enteral nutrition, with the total caloric and protein intakes set at 1500 Kcal and 70 g, respectively. Trace elements were added to this regimen. The wound was sprayed with a bFGF preparation and covered with a wound covering agent to maintain a moist environment. Since then, the wound underwent a marked reduction in size.

[Conclusion] It was believed that for the care of a decubitus ulcer, improving the patient's nutritional state and selecting appropriate localized care are important.

SYMPOSIUM I: ANGIOGENESIS, WOUND HEALING IN GI TRACT

MODERATED BY HIROSHI SHIMADA AND SHIGEHICO KAWAKAMI

THERAPEUTIC ANGIOGENESIS FOR ISCHEMIC LIMBS BY TRANSPLANTATION OF AUTOLOGOUS FRESH BONE MARROW

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Background: Previously, we succeeded regeneration of well-vascularized granulation tissue on wound beds of nonhealing leg ulcer by transplantation of autologous unfractionated fresh bone marrow.

Objective: We investigated efficacy and safety of autologous intramuscular implantation of unfractionated fresh bone-marrow cells in patients with ischemic limbs.

Material and Methods: Patients with arteriosclerosis obliterans (ASO) or thromboangitis obliterans (TAO) were eligible for the study if they had Fontaine II to IV ischemic limb despite best-tolerated medical therapy, no conventional revascularization treatment option. Twelve patients were injected with fresh bone marrow diluted twice by heparinized saline into gastrocnemius, soleus, and anterior tibialis anterior of the 15 ischemic limbs. Functional outcomes were assessed based on Fontaine classification.

Result: In 10 of 15 limbs (7 of 12 patients) Fontaine grade was significantly improved. Five limbs in 5 patients sustained foot ulcer, 3 of 5 limbs resulted in amputation. All of 10 improved limbs suffered arterial occlusion distal to popliteal artery, whereas limbs sustained ulcers suffered arterial occlusion proximal to femoral artery. No adverse event was observed.

Conclusion: Autologous intramuscular implantation of fresh bone-marrow cells could be safe and effective for achievement of therapeutic angiogenesis. Indication of this therapy may be limited to patients who suffered arteries distal to popliteal artery.

THERAPEUTIC ANGIOGENESIS INDUCED BY CONTROLLED RELEASE OF FIBROBLAST GROWTH FACTOR-2 FROM INJECTABLE CHITOSAN/NON-ANTICOAGULANT HEPARIN HYDROGEL IN A RAT HIND LIMB ISCHEMIA MODEL

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Aim: Addition of various heparinoids to the lactose-introduced, water-soluble chitosan (CH-LA) aqueous solution produces an injectable chitosan/heparinoid hydrogel. The purpose of this study was to examine the ability of the injected FGF-2/CH-LA/IO₄-heparin hydrogel to induce vascularization and fibrous tissue formation.

Methods: FGF-2/CH-LA/IO₄-heparin hydrogels (100 μ l of hydrogel consisting of 20 mg/ml of CH-LA, 2 mg/ml of IO₄-heparin, and 50 μ g/ml of FGF-2) was subcutaneously injected into the backs of mice or rats. Furthermore, The effect of percutaneous injection of FGF-2/CH-LA/IO₄-heparin hydrogel at eight sites (25 μ l per site) into ischemic left lower limbs produced by ligating the femoral artery of rats was examined from day 4 to at least day 28 postinjection.

Results: The injection of FGF-2/CH-LA/IO₄-heparin hydrogels into the backs of mice or rats resulted in significant increases in blood vessel formation, significant vascularization and fibrous tissue formation near the injection site. FGF-2/CH-LA/IO₄-heparin hydrogel into ischemic left lower limbs of rats also significantly recovered and increased blood flow, and blood oxygen in the calf and thigh.

Conclusions: These results indicate that the controlled release of biologically active FGF-2 molecules from FGF-2/CH-LA/IO₄-heparin induces angiogenesis and possibly collateral circulation in the ischemic limbs of rats.

RELATIONSHIP BETWEEN THE DEGREE OF OMENTAL GRANULATION TISSUE AND APOPTOSIS IN OMENTAL IMPLANTATION FOR PERFORATED ACETIC ACID-INDUCED GASTRIC ULCER IN RATS

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Aim: Omental implantation is 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. Omentum is thought to play an important role in the ulcer healing. To clarify the relationship between the degree of omental granulation tissue and apoptosis in omental implantation for perforated acetic acid-induced gastric ulcer in rats.

Methods: In one group of rats in which acetic acid-induced gastric ulcers were perforated and omental implantation was used for repaired. Animals were sacrificed, using an anesthetic overdose, 1, 3, 5, 10, 20, 30 days postsurgery. The tissue sections were stained with HE and TUNEL method.

Results: Histological assessment of the height of the omentum showed that the degree of the omental granulation tissue had increased by day 10. However, the width of the omental granulation tissue was not reduced. TUNEL positive cells were located only in the surface of the omental granulation tissue. TUNEL index increased rapidly from 1, peaking on day 5, in parallel with a marked the degree of omental granulation tissue.

Conclusions: These results indicated that apoptosis may lead effectively to granulation tissue formation and promote a scarless repair process.

UTILIZING VACUUM-ASSISTED CLOSURE (V.A.C.) AS ONE OF THE METHOD FOR WOUND BED PREPARATION (WBP)

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Aim: Treating intractable lower extremity ulcers are challengeable for us. We utilize Vacuum-Assisted Closure (V.A.C.) alternatively on various ischemic lower extremity ulcers as the method of Wound Bed Preparation and we received successful response from this method.

Methods: Before operation, the possibility of wound healing on ischemic foot ulcers are predicted by using Laserdopp PV2000 (Vasamedics, USA) which measures Skin Perfusion Pressure (SPP). If SPP value is less than 30 mmHg, arterial reconstruction is necessary procedure to rescue the ischemic foot. Before applying V.A.C., debridement of necrotic tissue was performed. V.A.C. therapy was utilized for the wounds didn't response to the other treatment. V.A.C. therapy is continued for 2 weeks to 1 month.

Results: Wound beds are well prepared by using V.A.C.

Conclusion: V.A.C. is less invasive method and can be used for the patient who is not candidate for operation.

SYMPOSIUM II: WOUND HEALING UNDER UNUSUAL CONDITIONS

MODERATED BY KIYOSHI KUBOCHI AND ICHIRO ONO

CONSIDERATION OF PATHOLOGIC CONDITIONS AND MULTIMODAL THERAPY FOR KELOIDS AND HYPERTROPHIC SCARS

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Aim: We have treated keloids and hypertrophic scars with multimodal therapy including postoperative electron-beam irradiation. The purpose of this study was to investigate the optimal dose of postoperative irradiation. Moreover, we reported about genetic analyses using cDNA microarray.

Methods: In clinical study, up to 2002, we treated all regions with 15Gy/tripartition/3days, but since 2003, we have used 20Gy/quadripartition/4days for the anterior chest and the scapular and suprapubic regions, 10Gy/bipartition/2days for the ear, and 15Gy/tripartition/3days for all other regions. In genetic analyses, three samples of keloids and normal skins were analyzed using cDNA microarray.

Results: In clinical study, the total recurrence rate was 28.9% (pre-2002) and 16.0% (post-2003). In genetic analyses, genes such as TGF-beta1, PDGF, IGF, NGF, TNF, and HSP47 were up-regulated on keloid samples.

Conclusions: The results suggest that keloid sites with a high risk of recurrence should be treated with 20Gy/quadripartition/4 days.

CHRONIC NONHEALING ULCERS TREATMENT AT WOUND CARE CENTER

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Summary: A wound care center is specialized in a medical treatment at outpatient basis for patients who are suffering from chronic wounds. Two percent of the general population in the U.S. is affected with chronic nonhealing wounds. Usually, chronic wounds need more than 1 year to be healed, otherwise these are unable to be healed completely. However, the wound care center enables those wounds to be healed in a short term, by using protocol driven, team care approach. In the U.S., it was reported that 80% of cases which had never been healed in more than 2 years were healed at the wound care center within 14 weeks. As recognized that such a wound care center is necessary also in Japan, we initiated a wound care center in January 2003, aiming to heal the chronic wounds. We reports the chronic nonhealing wounds treatment in our wound care center.

EFFECT OF ARTIFICIAL CO₂ FOOT BATHING ON WOUND HEALING, ESTIMATED BY A TWO-DIMENSION LASER DOPPLER FLOW-METRY

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Objective and Methods: The first part of this report is clinical study. Five patients with foot ulceration were treated by foot bathing in 35 °C water and in 35 °C CO₂ water (AS care™). After bathing, microcirculation of the foot was measured using laser Doppler, and comparison was made between water bathing and CO₂ bathing. The second part is in vivo experimental study using the ischemic limb model of mice. Bilateral femoral arteries were ligated and a full thickness skin defect in 2.5 mm diameter was created on each leg. A left leg was soaked in 35 °C water, and a right leg in 35 °C CO₂ water. Microcirculation of both legs was measured, immediately after bathing, and on 2nd and 4th day. Diameter of skin defect was measured on 2nd and 4th day. Comparison was made between left and right leg.

Results: In the first study, microcirculation of the foot was improved after CO₂ water bathing, compared with water bathing ($p < 0.05$). In the second study, the mean size of the skin defect on right leg (CO₂ bathing) was smaller than left, on both 2nd and 4th day ($p < 0.05$). However, there was no statistical difference in microcirculation between right legs and left legs.

Conclusion: The results of our study suggest that CO₂ water bathing treatment improves microcirculation and promotes wound healing process.

EFFECT OF MARROW MESENCHYMAL STEM CELLS ON CUTANEOUS WOUND HEALING OF PIGS

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Aim: We reported cutaneous wounds of rats healed with less scar by transplanting mesenchymal stem cells. There was a possibility that the cells responded to the wound healing and regenerated dermal structure nearly normally. In this study, we examined about whether or not scar forming was in the same way restrained at the pigskin which structure resembles humans.

Methods: Bone marrow of the pelvis of 4 week L.W.D. pig was suspended into culture medium and plated on a plastic dish. Three to four passaged plastic adhesive cells were used as mesenchymal stem cell (MSC). MSCs were autografted into the 2 cm length cutaneous wounds of back of pigs. The wounds were harvested 4 weeks after operation and investigated macroscopically and histologically.

Results: The wounds transplanted MSCs were healed faster and with less scar than control wounds macroscopically. The wounds transplanted MSCs showed thick and well-oriented collagen architecture than control wounds histologically.

Conclusions: These results indicate that autografted pig's MSCs can respond quite normally to wound healing and regenerate dermal structure. We are going to apply these results to the human clinically for scarless wound healing.

SYMPOSIUM III (1): TISSUE REGENERATION AND WOUND HEALING MODERATED BY TATSUO NAKAJIMA AND TETSUO OHTA

BASIC FIBROBLAST GROWTH FACTOR REDUCES SCAR FORMATION THROUGH APOPTOSIS IN INCISIONAL WOUNDS AND OPEN WOUNDS

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The present study examined the relationship between the degree of scar formation and the level of apoptosis in both rat skin incisional and open wounds after treatment with bFGF (1 µg/cm of wound). In incisional wounds, the degree of granulation formation in the bFGF-treated group significantly decreased on days 14 and 28, in parallel with a significantly persistent increase in the number of apoptotic cells over the first 14 days. In open wounds treated with implanted collagen gels, significant inhibition of wound contraction was observed only in implanted bFGF-treated collagen gels on day 28, in parallel with a significantly increase in the number of apoptotic cells on day 14. These findings strongly suggest that the accelerated apoptosis in the bFGF-treated wounds contributes to the decreased cellularity in granulation tissues through elimination of cells with apoptosis, which resulted in the reduction of scar formation and inhibition of wound contraction.

BIOCHEMICAL STUDY ON CONDITIONS FOR TRANSPORTATION OF AUTOLOGOUS CULTURED DERMAL SUBSTITUTE BY EVALUATING FIBROBLASTS AND VEGF PRODUCTION

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Purpose: Autologous cultured dermal substitute (CDS) can be used in the treatment of children with burn scarring or giant pigmentous nevi. Transplanted CDS needs to be preserved when surgery is postponed owing to the recipient's health condition. This study aimed to investigate the optimum conditions for preserving autologous CDS.

Method: Autologous CDS is manufactured by cultivating autologous fibroblasts on a two-layer spongy matrix of hyaluronic acid and atelo-collagen for 1 week. CDS is sealed in a bottle containing culture medium and is then transported to the hospital. Quantitative analysis was performed by measuring the amount of vascular endothelial growth factor (VEGF) using ELISA and the viability of fibroblasts in CDS using MTT assay over a period of 2 weeks under of different temperature conditions.

Results: At 37 °C, both viability and VEGF production of fibroblasts showed high values for 1 week but decreased after this period. At 25 °C, both viability and VEGF production of fibroblasts showed high values over a period of 2 weeks. At 4 °C, the potency of fibroblasts decreased markedly over time.

Conclusion: The results obtained in this study indicate that the potency of autologous CDS is maintained at 25 °C. This is advantageous for practical use of autologous CDS.

EVALUATION OF POTENCY OF MASS-PRODUCED ALLOGENEIC CULTURED DERMAL SUBSTITUTE BY MEASURING VEGF LEVELS

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Aim: As part of a Regenerating Medical Millennium Project of the Ministry of Health, Labor and Welfare, multicenter clinical research using allogeneic cultured dermal substitute (CDS) is being conducted. We have established a banking system in which CDS is cryopreserved and transferred in a frozen state to hospitals across Japan. The present study was designed to investigate the potency of cells to proliferate and release VEGF when CDS is manufactured using a repeated procedure.

Method: CDS is manufactured by seeding fibroblasts onto a two-layered spongy matrix composed of hyaluronic acid and atelo-collagen. Cryopreserved fibroblasts were defrosted and cultured, and 3/4 of the obtained cells were seeded onto the matrix to prepare CDS, while the remainder was again cultured in flasks. CDS was manufactured by repeatedly performing similar operations every week.

Results: It is necessary to examine how many times this operation can be repeated in the mass production of CDS. The results of this study indicate that the potency of cells to proliferate and release VEGF is maintained nine repeats of this procedure.

Conclusion: The fibroblasts derived from 1 cm² of skin a young donor have proven to have the potency to manufacture about 1000 sheets of CDS (10 × 10 cm), although the potency depends on the area of skin collection and the age of the donor.

EVALUATION OF SUTURELESS SKIN GRAFTING PROCEDURE USING PHOTOCROSSLINKABLE CHITOSAN (AZ-CH-LA)

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Aim: Az-CH-LA is an aqueous solution converted into an insoluble flexible hydrogel after short ultraviolet irradiation. It has entered the limelight as a wound dressing and tissue adherent excelling in biocompatibility, anti-infective activity and the ability to accelerate wound healing. In this study, we evaluated sutureless skin grafting procedure using AZ-CH-LA.

Methods: We made 3 cm × 3 cm skin defect with full-thickness and grafted 5 mm diameter auto-skin grafts by four procedures such as not fixed (A), sutured (B), adhered by cyanoacrylamide (C), adhered by Az-CH-LA in PBS (D) followed up to 8 days. These procedures were evaluated by the graft take rate (day 5) and by histological examination (day 8). The graft take rate was calculated by the equation (the number of grafts survived on day 5/ the number of grafts on day 0).

Results: The time required for each procedure was B > D > C > A. The graft take rates of B, C and D were almost 100% and significantly higher than that of A. Despite high take rate, epithelialization and granulation were impaired in C. In D, the granulation was significantly promoted but the epithelialization was slightly impaired. Then, we changed the diluent of AZ-CH-LA from PBS to DMEM/F12. By using DMEM/F12, epithelialization and granulation were significantly promoted.

Conclusion: Sutureless skin grafting procedure using AZ-CH-LA saves the effort of suturing and achieves excellent take rate as well as suturing. Moreover, DMEM/F12 was considered to be an appropriate diluent for AZ-CH-LA.

CULTURED DERMIS SEEDED WITH STEM CELLS DERIVED FROM ADIPOSE TISSUES

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Aim: Recently, cultured dermis with homologous fibroblasts seeded in collagen sponge has been clinically applied. We attempted to produce cultured dermis by seeding stem cells derived from adipose tissues in collagen sponge.

Methods: Cell masses containing stem cells, which were collected from inguinal adipose tissues in F344 inbred rats, were cultured, and cultured dermis was produced by seeding the cells in collagen sponge. Full-thickness skin-deficient layers were produced on the back of inbred rats, and an intractable ulcer model was produced by applying mitomycin C. The cultured skin was transplanted on the back of the intractable ulcer model rats, and skin samples collected later from the transplanted area were histologically examined.

Results: The cultured skin transplanted in the intractable ulcer model took satisfactory, and the granulation was better with the cultured skin than with collagen sponge alone.

Conclusions: We aim to produce cultured dermis using stem cells derived from human autologous adipose cells. The cultured dermis produced in this study took after transplantation, suggesting its greater applicability than cultured dermis produced using cells of the same species for the treatment of intractable ulcer.

ANALYSIS OF SELF-RENEWAL MECHANISM OF ES CELLS

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Aim: Embryonic stem (ES) cells have an ability to differentiate into a variety of cells. For future application of ES cells in cell therapy, we have been trying to clarify the mechanism of their self-renewal, which would lead to establishment of an expansion system. Since we found previously that STAT3 activation is sufficient for the self-renewal of mouse ES cells (1), we searched for a STAT3 target gene(s). We also tried to produce insulin-secreting cells from ES cells.

Methods: For identification of a STAT3 target, total RNA was prepared from ES cells, where STAT3 is either activated or inactivated, and subjected to DNA microarray analysis. For production of insulin-secreting cells, we introduced into ES cells the plasmid carrying insulin promoter sequence followed by green fluorescent protein (GFP) gene, and allowed them to differentiate.

Results: We identified embryonic ectoderm development (eed) as a STAT3 target. Our attempt to establish eed-null ES cells has failed so far. As for insulin-secreting cells, we could obtain GFP-positive cells from ES cells, though with low efficiency, after embryoid body formation.

Conclusions: Our data suggest that Eed might play an important role in maintenance of ES self-renewal. We also present the data that ES cells could be a useful resource for insulin-secreting cells.

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**SYMPOSIUM III (2): TISSUE REGENERATION AND WOUND HEALING
MODERATED BY MOTOHIRO NOZAKI AND KOICHI HIRATA**

SURGICAL PROCEDURES IN CONJUNCTION WITH THERAPEUTIC ANGIOGENESIS USING BONE MARROW CELL IMPLANTATION FOR ISCHEMIC LIMB GANGRENE

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Introduction: We reviewed the clinical outcome of surgical procedures in conjunction with therapeutic angiogenesis using autologous bone marrow cell implantation for ischemic limb gangrene.

Patients and Methods: Thirteen of our consecutive cases that were treated with those combined procedures were reviewed retrospectively. Under the general anesthesia, autologous bone marrow stromal cells were harvested, immediately processed in order to isolate cell suspension including monocytes, CD34 (+) cells, endothelial progenitor cells, etc., followed by injected intramuscularly into the ischemic limb. Minimal surgical debridement was carried out simultaneously. Additional surgery including skin grafting and direct suture was performed if necessary.

Results: Surgical procedures applied in this series included; (1) toe amputation in 12 cases, (2) allogeneic cultured dermal substitute application in 6 cases, (3) bone marrow cell impregnated collagen sponge application in 5 cases, (4) skin grafting in 8 cases, and (5) direct suture in 1 case. Eleven out of 13 cases required staged operations. Two lower limbs were eventually amputated at below knee level due to uncontrolled infection.

Conclusions: These combined therapy contributed to the survival of unreconstructable ischemic extremities. Patients' overall satisfaction was achieved because of avoiding major amputation.

APPLICATION OF HUMAN MESENCHYMA STEM CELLS TO REGENERATIVE WOUND HEALING

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Further detail analysis of human mesenchymal stem cells (hMSCs) to regenerative wound-healing model. The hMSCs are homogenous, of euchromatin nuclei and contain the developed cytoplasm in the maintenance medium. Longer than 4-day serum-free culture, cells turn into apoptosis with bone morphogenetic protein-2 alone, while with basic fibroblast growth factor (bFGF), the cells remain small and immature condition. The bFGF concentration of 0.25 to 25 μ g did not change this cellular morphology. In dual chamber chemotactic migration assay with 8 μ m-pore, there was significant hMSC migration was observed when human epidermal keratinocytes were placed in the lower chamber in 16-hour period than when human dermal fibroblasts or artery endothelial cells ($p < 0.01$). The hMSCs demonstrated the fusiform morphological changes when the direct co-culture with human keratinocytes was performed. Also, there was cell-to-cell adhesion observed in between two cell types. Therefore, the hMSCs are able to mostly migrate, proliferate and affect the cutaneous wound healing.

EXPRESSION AND REGULATION BY PITUITARY TUMOR TRANSFORMING GENE (PTTG) IN A LIVER REGENERATION

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Pituitary tumor transforming gene (PTTG), homologous to a mammalian securin, plays a pivotal role in cell transformation, however, its biological function(s) in normal tissues is not fully understood. Additionally, the tissue differentiation by cell transformation is not further understood yet. Therefore, a liver regeneration was sought to investigate the mechanisms of the regeneration. Rat hepatic PTTG mRNA was induced during the cell proliferative period of the liver regeneration both in vitro and in vivo. PTTG expression of the rat primary hepatocyte was stimulated by HGF in a dose dependent manner, and was suppressed when hepatocyte proliferation was inhibited by TGF- β 1. A positive PTTG immunoreactive co-localizing with BrdU in the hepatocyte nucleus was found and there was a concurrent sister chromatid itself by the immunofluorescent labeling of PTTG with CK18. Since the correlation of PTTG mRNA expression and cell proliferation are also observed in primary rat cultured hepatocytes, PTTG may be novel marker of cell proliferation both in vitro and in vivo liver regeneration.

THE ANALYSIS OF THE DIFFERENCE OF EXPRESSION GENES BEFORE AND AFTER THE TRANSITION IN FETAL WOUND HEALING USING GENECHIP

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Aim: We reported that complete regeneration can be observed when the fetus are wounded at embryonic day (E)13, but not in E14 in mice.

We compared the differences of expression genes that are expressed on E13 wound healing and E14 in mice using Genechip (Affimetrix, Murine Genome U74A).

Methods: The wounded skin were obtained at 14 hours after wounding as a lot of genes were changed in expression during fetal wound healing around this time point. Each gene expressed at E13 and E14 wounds was subtracted from the contrary intact skin, and next compared the difference of genes that expressed in E13 or E14 wound healing.

Results: We found 11 genes that expressed in E13 wound healing, but not in E14 wound healing. And 12 genes that expressed in E14 wound healing but not in E13.

Conclusions: Genechip was thought to be a powerful tool to examine the mechanism of fetal wound healing.

LONG-TERM OBSERVATION OF THE TISSUE-ENGINEERED HUMAN EAR-SHAPED CARTILAGE IN SPECIAL REFERENCE TO MECHANICAL PROPERTY

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We cultured chondrocytes that were harvested from four different sites (nasoseptal, articular, costal, and auricular cartilage), which were subsequently seeded onto biodegradable poly (LA-CL, 50:50) polymer. Chondrocyte-polymer composites were implanted in vivo, and then analyzed for long-term observation that includes gross morphology, histology, and mechanical property. Results on gross morphology revealed that all tissue-engineered cartilages retained human ear shape for up to 40 weeks in vivo. Such tissue-engineered cartilages as nasoseptal, articular, and auricular origin demonstrated similar mechanical property with the native bovine auricular cartilage. On the contrary, tissue-engineered cartilage of costal origin developed mineralization and showed remarkably rigid mechanical property, which is indicative of probable stem cells in cartilage structure.

Result of mechanical testing

Bovine Auricle Cartilage	Nasoseptal	Articular	Costal	Auricular
25.18 ± 0.27	10 week 4.51 ± 0.04**	5.75 ± 0.32**	18.42 ± 0.56**	4.82 ± 0.18**
	40 week 14.15 ± 0.20**	21.71 ± 1.08*	43.83 ± 1.78**	24.62 ± 0.92

*p < 0.05, significantly different from bovine auricle cartilage

**p < 0.01, significantly different from bovine auricle cartilage

All samples were n = 3, Unit: N

These results lead us to conclude that tissue-engineered cartilages of nasoseptal, articular, and auricular origin were appropriate sites as cell sources as they retained three dimensional shape of the human ear with the restoration of original mechanical property, while that of costal origin further needed to regulate the mineralization process.

REGENERATION OF WOUND BED ON NONHEALING LEG ULCERS BY TRANSPLANTATION OF AUTOLOGOUS FRESH BONE MARROW -HISTOPATHOLOGICAL STUDY

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Background: Previously, we reported our successful treatment of nonhealing leg ulcer by transplantation of autologous unfractionated bone marrow-impregnated collagen sponge on debrided wound. Although this procedure dramatically recovered disrupted wound healing of nonhealing ulcers, its pathological study has not been elucidated.

Objective: To investigate histopathological changes between wound bed of nonhealing leg ulcer (disrupted wound bed) and well-granulated wound bed (regenerated wound bed) formed by bone marrow transplantation in a same lesion of a same individual.

Material and Methods: Nonhealing leg ulcers over five-year history despite appropriate wound care were involved in this study. The specimen of disrupted wound bed was collected in the surgically debrided tissue of nonhealing leg ulcer for preparation of bone marrow transplantation. The specimen of regenerated wound bed after bone marrow transplantation was collected in the second operation for closing the wound by skin graft. Formalin-fixed, paraffin-embedded specimens were stained immunohistochemically with anti-CD31 antigen for endothelial cell and α -smooth muscle actin antibody for pericyte and myofibroblast as well as hematoxylin and eosin staining.

Result: In the regenerated wound bed group, neovascularity in loose matrix without myofibroblast is prominent in the puter layer of granulation tissue. In its surfacemost layer, naked endothelial cell tubes without pericyte lining, characteristic of neoangiogenesis, are observed. Myofibroblasts with collagen bundles are observed in the basal layer of granulation. In the disrupted wound bed, vessels with small diameter are situated between scar tissue with myofibroblast proliferation. In its surface layer, vessels are covered with myofibroblast and no naked endothelial cell tube is found.

Conclusion: Granulation tissue on the wound bed after bone marrow transplantation is a mass of regenerated vessels neovascularizing towards wound surface, whereas in wound bed of nonhealing leg ulcer, angiogenesis is disrupted. Proliferated morbid myofibroblasts in the disrupted wound bed may be caused by longstanding chronic inflammation due to stasis.

SYMPOSIUM IV: UPDATE OF PRESSURE ULCER TREATMENT MODERATED BY SADAO ANAZAWA AND YOKO YOSHIMURA

PRESSURE ULCER TREATMENT WITH KITCHEN WRAP

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Introduction: From 2003, covering of wounds with kitchen wrap has been done at this hospital as localized treatment for pressure ulcers (denoted here as wrap therapy). The therapeutic action of this method is remarkable, and it has become the first choice for treatment of pressure ulcers. Here, we have indicated the realities of wrap therapy at this hospital and we have reported on comparison of this method to conventional dressings.

Wrap therapy: No disinfection was done; the wound was irrigated with tap water and covered with kitchen wrap. Necrotic tissue was actively debrided, and frequent irrigation was done for pressure ulcers with a large amount of exudate. Systemic administration of antibiotics was performed for infected pressure ulcers.

Comparison with conventional dressings: Based on the sum of DGN in DE-SIGN (depth, exudate, size, inflammation/infection, granulation, and necrosis) evaluation, we compared the therapeutic action after 2 weeks for a group (15 cases) treated with a commercially available dressing (Duoactive ET, Hydro-site, etc.) and a group treated with wrap therapy (15 cases).

Results: The DGN sum for commercially available dressings (15 sites in 15 cases) went from 3.2 ± 2.6 beforehand to 2.1 ± 3.1 after 2 weeks; that for wrap therapy (18 sites in 15 cases) went from 5.5 ± 2.6 beforehand to 2.7 ± 1.6. A significant difference in before and after scores was noted.

Discussion: Advantages of wrap therapy are that treatment is extremely simple, reducing the burden on staff, that it heightens motivation in handling pressure ulcers, which is linked to staff confidence – i.e., “it can be used to treat severe pressure ulcers as well” – because of its exceptional therapeutic action, and that the treatment is obviously economical.

THE MANAGEMENT OF THE CARRY IN PRESSURE ULCERS IN OUR HOSPITAL

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Aim: We report that we have the good results for the incurable pressure ulcers on setting at home or other hospitals, by using a series of therapies in our hospital.

Methods: For the carry in pressure ulcers, We chose the therapy by using the F (Fukai) scale, which is used in our hospital for evaluation of pressure ulcer therapy. The conservative therapy, local wound treatment (e.g., ointment, occlusive dressing, etc.) and therotherapy and OHP, was done and evaluated the therapy continuously.

As conservative therapy could not obtain the effectiveness, then we introduced the surgical approach.

Results: In eight cases, the complete wound healing were seven, improvement was one. The only one case was healed by conservative therapy, others were selected the operative therapy, but all of them were combined the conservative therapies.

Conclusions: It was very difficult healing pressure ulcers which were on setting their at home or other hospitals, because ineffective debridement and nonsystematic selection of drugs for local wound treatment. The results of this report suggest that incurable pressure ulcers needed by using a series of therapies early and as many as possible.

INTRA-WOUND DETECTION OF BACTERIA BEFORE AND AFTER PRESSURE SORE SURGERY

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Aim: Bacteria can be detected in the wound in many instances of pressure sores. The current research performed adequate debridement while taking precautions for surgical field contamination and studied whether or not bacteria could be detected in the wound immediately after surgery for closed-wound pressure sores.

Methods: Subjects were five cases of pressure sores underwent a perforator flap. Povidone-iodine was used for disinfection during surgery; a technique was implemented so as not to have the surgical instruments or surgeon come in direct contact with the surface of the pressure sore during surgery. Immediately following surgery, culturing of bacteria in the suture wound was performed.

Results: Multiple strains of bacteria were detected in preoperative bacterial tests for the four cases; one or more strains of bacteria were detected in post-operative tests as well. All of the cases in the current research had a course that subsequently proceeded smoothly with no symptoms of infection and even today, several months to several years after surgery, no problems have been noted.

Conclusions: Thorough contamination prevention measures are considered unnecessary. Instead, some degree of contamination will naturally occur, but performing thorough debridement and preventing the onset of infection by covering the wound with tissue that has adequate blood flow are considered vital.

USEFULNESS OF FOOD WRAP FILM FOR TREATING PRESSURE ULCERS

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Aim: In 2000, Toriyabe and Suemaru¹⁾ reported a novel method for treating decubiti using food wrap film (Saran Wrap[®]). We applied this simple technique (wrap therapy) to a total of 118 pressure ulcers in 90 patients aged from 32 to 101 years old (mean: 67).

Methods: Fixation of the wrap film by a nonwoven fabric tape assisted at draining the inflammatory exudates.

Results: Necrotic crusts disappeared within 17 weeks, and undermining pockets disappeared within 24 weeks. All of the 118 ulcers were healed (epithelialized) within 39 weeks, clinically confirming the effectiveness of the wrap therapy.

Consideration: The wrap coating contributed to the maintenance of the moist environment in the lesions, resulting in the exudation of polymorphonuclear leukocytes and macrophages²⁾³⁾. This process functioned as defense mechanisms against bacterial growth and toward the absorption of necrotic debris.

Conclusions: The wrap therapy was applicable to treating advanced ulcers (grade 3 or 4 after the NPUAP⁴⁾ classification), pocket-forming deep ulcers, or decubiti with bacterial infection or extensive inflammatory exudation. The procedures were convenient and time- and cost-saving. It was indicated that the food wrap film was useful as a new vulnerary-covering material.

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USEFULNESS OF HYDROCOLLID DRESSING FOR THE EARLY-STAGE PRESSURE ULCER WITH NECROSIS

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The surfaces of early stage pressure ulcers, pressure ulcers with short history, are very often covered by necrotic tissue. They include stage II ulcers and the pressure ulcers could restore their dermis.

Methods: We used hydrocollid dressings for the early stage pressure ulcers with necrosis, those were clinically diagnosed as stage IIs or stage IIIs. We evaluated the benefit of our treatments for a period of 3.5 year.

Results: Forty eight early stage pressure ulcers with necrosis were initially treated with hydrocolloid dressings. After an average of 16 days, the ulcers were reassessed as 13 stage II, 34 stage III, and one stage IV pressure ulcers. All 13 stage II ulcers started to epithelialize. 29 of 34 stage III ulcers had started to epithelize. But three ulcers developed hard eschars, and two ulcers became infected. One stage IV ulcer started to epithelize.

Conclusions: It is not easy to assess stage II and III pressure ulcer with necrosis is. If a stage II ulcer is treated with dry dressing, the necrosis might become deeper and the ulcer could become stage III.

Overall outcomes of our treatments for necrotic pressure ulcers under occlusive methods showed that 89.6% of ulcers started to epithelize after an average of 16 days of treatment. Dressing changes were done every day or every other day. The infection rate was as low as 4.2%. Hydrocolloid dressings promote autolysis of necrotic tissues of early stage pressure ulcers, and then it accelerates the wound healing.

ANALYSIS OF PORTAL PRESSURE AND TYPE-1 PLASMINOGEN ACTIVATOR IN THE EXCESSIVE HEPATECTOMY

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Aim: In hepatectomy and liver transplantation, it had been reported that post-operative excessive portal hypertension is a cause of hepatic failure. The purpose of this study is to examine the correlation with portal pressure and PAI-1 which plays an important role in the priming phase of the liver proliferation by using hepatic failure rat model.

Methods: In our findings, the 1-week survival rates of rats in 90% hepatectomy group were all 100%, but all rats in the 95% hepatectomy group died within 96h. Therefore, we used 90% group as an acceptable hepatectomy model and 95% group as a hepatic failure model. Portal pressures were measured by a transducer after a 27G needle had been inserted directly into the portal vein. Hepatic gene expression of PAI-1 was examined by real time PCR.

Results: The portal pressure of 95% group was significantly higher than that of 90% group. PAI-1 mRNA levels were significantly increased in 95% group compared with those in 90% group on 5 hr after hepatectomy. Moreover portal pressures were intercorrelate with PAI-1 mRNA levels.

Conclusions: It is suggested that overexpression of PAI-1 which was induced by excessive portal hypertension, is one of the reason of liver failure after massive hepatectomy

INCISION OF SUBCUTANEOUS POCKETS OF DECUBITUS ULCERS

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Aim: To treat decubitus ulcers, we usually remove the necrotic tissue surgically and/or nonsurgically. The wound should heal by wound contraction and epithelization. However, a subcutaneous pocket can remain despite apparently appropriate treatment.

Methods: After precise information about the operation was given to the patients and their families, and consent received, anesthetics were injected locally. Incision was made as a first by a #15 scalpel on the skin of the deepest pocket

and then it was cut out at full thickness using an electric scalpel to make an open wound. An additional incision was made to cut open another overlapping area. Hemostasis was done by ligation, electrocoagulation and hemostatic spongy jel application. A thick dry gauze over the wound was fixed by elastic bandages. Numbers of incised areas depended on each decubitus ulcer.

Results: The numbers of incisions ranged from two to seven, depending on the depth and shape of the pocket of the decubitus ulcer.

Conclusions: We could see and clean the deepest portion of the decubitus ulcer clearly after these incisions. Although we have no case of total healing in the 4 months since we started this series, the size of the wound decreased in all cases, and review of the healing process gives grounds for optimism concerning eventual outcome. We think this multiple incision method is effective for the re-starting the wound healing.