



Abstracts

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Congress President: Norio Kumagai, M.D. Professor, Department of Plastic and Reconstructive Surgery,
St. Marianna University School of Medicine

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

GENERAL I; TISSUE REGENERATION 1

TISSUE-ENGINEERED ORAL MUCOSA EQUIVALENTS TO CLINICAL TRIALS

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Aim: Evaluation of clinical trial of human ex vivo-produced oral mucosal equivalents (EVPOMEs).

Methods: Culture of human oral mucosa keratinocytes and EVPOMEs were generated in a serum-free culture system without the use of an irradiated xenogeneic feeder layer, as described previously below. We operated with EVPOMEs for 100 clinical cases with various oral mucosal defects.

Results: The grafted tissues have taken well in 84 cases, and not utilized in 16 cases because of some reasons. The EVPOMEs is handy and pliable to grasp, and feasible to suture onto a wound bed.

Conclusions: The EVPOMEs should be one of the most useful materials for the epithelial grafting on oral mucosal defects in the future.

References:

1. Izumi K, Takacs G, Terashi H, et al: J. Oral Maxillofac. Surg., 57, 571–1999.
2. Izumi K, Terashi H, Marcelo CL, et al: J Dent Res, 79–798, 2000.
3. Izumi K, Terashi H, Marcelo CL, et al: Tissue Eng, 9:163, 2003.

EXAMINATION OF ADULT HEPATIC BIPOTENT PROGENITOR CELLS IN RAT REGENERATING LIVER AFTER PARTIAL HEPATECTOMY

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Background/Aims: It remains unknown whether normal adult liver contains bipotent stem/progenitor cells and, if it does, how severe damage activates them to proliferate. The aim of this study was to clarify whether normal adult liver contains hepatic stem/progenitor cells and, if present, whether they are activated by extensive hepatectomy.

Methods: Adult rat liver cells were isolated and cultured at low cell density, and colony-forming assay was performed to evaluate cell proliferation capacity. Immunocytochemistry and reverse-transcription polymerase chain reaction (RT-PCR) were used to investigate multilineage differentiation capacity. The proportion of colony formation by the cells of normal liver and regenerating liver after 70% or 90% partial hepatectomy (PH) was compared to determine whether progenitor activation is induced by PH.

Results: Only a few epithelial colonies ($0.043 \pm 0.009\%$ of non-parenchymal cells, $n = 9$) continued to proliferate more than one month in vitro. The proportion of progenitor colonies was greater in the non-parenchymal fraction. RT-PCR and immunocytochemistry showed that these progenitor colonies expressed both hepatocyte and cholangiocyte markers. The proportion of progenitor cells that formed bipotential, and long-term colonies did not differ significantly between normal and PH liver.

Conclusions: Adult normal liver contains bipotent hepatic progenitor cells, but they are not activated even after extensive hepatectomy.

THE DIFFERENCE BETWEEN IN VITRO AND IN VIVO FETAL WOUND HEALING IN MICE

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Aim: Fetal cutaneous wounds made at proper developmental stages regenerate completely. Thus fetal wound healing can be a mammalian regeneration model. On the contrary, the technique to make wounds and to manipulate the wounds are difficult. Ihara et al. showed the correlation between in vivo fetal wound healing and in vitro fetal wound healing using tissue culture model. This in vitro model is a useful model of fetal wound healing, and is frequently used now a days.

Methods: Ihara used rats as a model. If we follow Ihara's model for mice, we found it very difficult because of the fragility of the skin. Thus we modified their technique.

Results: Our modified tissue culture technique using mice fetuses worked well, and could get the similar results as Ihara.

Conclusion: As mice are a useful animal to research the molecular mechanism of regeneration, in vitro wound healing model is essential for massive analysis.

GENERAL II; TISSUE REGENERATION 2

EFFECT OF INCHIN-KO-TO (ICKT) ON HEPATIC REGENERATION

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Background and Aim: Inchin-ko-to (ICKT) is an herbal medicine that is known to inhibit hepatocyte apoptosis in a hepatitis model as well as improve the hepatic function by promoting the secretion and excretion of bile. We examined the effect of ICKT on hepatic regeneration using the extensive hepatectomy model.

Methods: Male Wistar rats orally received 2g/kg of ICKT from 3 days preoperatively and underwent 70% or 90% hepatectomy.

Results: In the 70% hepatectomy model, the remnant liver/body weight ratio had increased from 12 hours postoperatively in the ICKT group with a significant difference compared with the control group. The 24-hr postoperative ratio was $2.3 \pm 0.16\%$ in the ICKT group and $1.7 \pm 0.08\%$ in the control group, and increased 1.35-fold in the ICKT group with a significant difference compared with the control group. There remained no significant difference in the liver/body weight ratios at 96 hours postoperatively or later between both groups. In the 90% hepatectomy model, the remnant liver/body weight ratio had increased until 24 hours postoperatively with no significant difference between both groups, but the 48-hr postoperative ratio was $1.7 \pm 0.21\%$ in the ICKT group and $1.2 \pm 0.12\%$ in the control group and increased 1.5-fold in the ICKT group with a significant difference compared with the control group. However, as in the 70% hepatectomy model, there was no significant difference in the liver/body weight ratios at 96 hours postoperatively or later between both groups.

Conclusions: ICKT promoted liver regeneration after massive hepatectomy early phase.

References:

1. Masahiro Yamamoto et al. Genipin, a Metabolite Derived From the Herbal Medicine Inchin-ko-to, and Suppression of Fas-Induced Lethal Liver Apoptosis in Mice. Gastroenterology; 118:380–389, 2000.
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BONE MARROW-DERIVED STROMAL CELLS APPEAR IN THE REGENERATING EPIDERMIS AFTER DEEP DERMAL BURN INJURY

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Aim: Recent studies have shown that bone marrow-derived cells have an important role in the wound healing process. However, the characteristics and functional roles of these cells in the wound healing sequence are poorly understood. Here, we investigated the cellular kinetics of bone marrow stromal cells in deep dermal burn wounds.

Methods: Bone-marrow stromal cells were collected from male Lewis rat femurs. The isolated bone marrow cells were cultured in minimum essential medium-alpha medium (α -MEM; Invitrogen) containing 10% fetal bovine serum. After cultured for a week, adherent cells were labeled with PKH-26 fluorescent marker. These cells were then transfused into recipient Lewis rats where they homed to the bone marrow. Five days after transplantation, a deep dermal burn was made on the back of the rat; the wound was biopsied at day 7, 10 and 14 post-burn.

Results: PKH-positive cells were found mainly in the upper dermis of the burn injury closely beneath the regenerating epidermis on day 14. PKH-positive cells were characterized by immunohistochemical staining for CD34, CD105, cytokeratin, and vimentin. The PKH-positive cells, stained only for vimentin, indicating fibroblast characteristics.

Conclusion: It is suggested that the fibroblast-like bone-marrow stromal cells appear beneath the regenerating epidermis after burn.

A NEW METHOD OF CULTURED FLAP PREPARATION

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Aim: Cultured epidermal sheet and bilayered cultured skin have been used clinically but they lack subcutaneous tissue. The aim of this study is to improve the cultured skin and produce epidermis, dermis, and subcutaneous tissue (adipose tissue) simultaneously in vivo.

Methods: We disseminated mesenchymal stem cells from adipose tissue on one side of collagen sponges at the density of 1.0×10^5 cells/cm² and incubated overnight. Then we turned over these sponges and disseminated dermal fibroblasts and keratinocytes at the density of 1.0×10^6 cells/cm² on the other side of sponges. Then we cultured for one week and implanted on the backs of SCID mice with or without basic FGF. Six weeks after implantation, specimens were taken.

Results: Epidermis, dermis and adipose tissue were formed in the bFGF applied group.

Conclusions: This cultured flap was thicker than cultured skin and could be a choice of flap operation.

GENERAL III; TISSUE REGENERATION 3

WHEN DO HAIR FOLLICLE CELLS TRANSFORM TO EPIDERMAL KERATINOCYTES DURING EPITHELIALIZATION?

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Introduction: In 2nd degree burn and split-thickness donor site, the epithelialization derived from the residual hair follicle cells occurs. It promotes better wound healing to transform hair follicle cells to epidermal keratinocytes well.

Methods: As the fundamental experiment, we analyzed in vivo and in vitro cell membranous fatty acid composition of hair follicle cells and epidermal keratinocytes, and studied when the former transformed to the latter during epithelialization. The in vivo epithelialization model was made from culture method of the outer root sheath cells, which we developed before. Lipid component was extracted with Folch extraction method and the extracted phospholipid component was fractioned in thin-layer chromatography, and the fatty acids were methylated before being identified and quantified into gas chromatography, and then 19 kinds of fatty acid were shown in percentage.

Results: Membrane fatty acid composition of in vivo hair follicle cells was greatly different from that of in vivo epidermal keratinocytes, and in vitro of the outer root sheath cells, which was in vivo epithelialization model, it became the same composition as that of in vitro epidermal keratinocytes by the incubation three weeks.

Conclusions: The hair follicle cells can transform to the epidermal keratinocytes in the middle of epithelialization by three weeks.

PLASMINOGEN ACTIVATOR INHIBITOR-1 PLAYS AN IMPORTANT ROLE IN LIVER FAILURE AFTER EXCESSIVE HEPATECTOMY IN THE RAT

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Aim: Fibrinolytic factors, are thought to be implicated in the regulation of liver regeneration. To clarify the role of these factors in fatal liver failure after excessive hepatectomy, we determined whether angiotensin-converting enzyme (ACE) inhibition could improve the survival rate of rats undergoing 95% partial hepatectomy (PHx).

Methods: Wistar rats were divided into three groups: group 1, 90% PHx; group 2, 95% PHx; group 3, 95% PHx treated with the ACE inhibitor (5 mg/kg/day), before PHx. Using liver tissues collected after PHx, RT-PCR was used to determine the quantitative changes in the expression of PAI-1 and uPA mRNA. Hepatic PAI-1 protein and uPA activities were determined by ELISA. To evaluate the effects of ACE inhibitor, survival studies and Ki-67 immunohistochemistry were performed.

Results: Although the PAI-1 mRNA content and the hepatic PAI-1 protein content in group 2 continued to be elevated, the uPA activity in group 2 was decreased when compared with group 1. Additionally, the hepatic PAI-1 level was decreased and the survival rate was improved in group 3 compared to group 2.

Conclusions: This study addresses an important role of PAI-1 in the early stage of liver failure after excessive hepatectomy, via maturation of pro-uPA and supports the concept that fibrinolytic factors are possible molecular targets for therapeutic strategy.

GENERAL IV; WOUND MANAGEMENT

CLINICAL EFFECTS OF CARBOXYMETHYLCELLULOSE (AQUACEL[®]) FOR THE TREATMENTS OF PARTIAL THICKNESS BURNS

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Aim: Carboxymethylcellulose (AQUACEL[®]) is indicated for the treatment of skin wounds, including partial thickness burns. It has a strong capacity for absorption and transportation of fluid and bacteria-retaining ability. In order to evaluate the clinical effects, a non-comparative prospective study in partial thickness burns was initiated.

Methods: In this study 33 patients with partial thickness burns were included. The mean size of the wounds treated with carboxymethylcellulose was 2.0% body surface area (0.2~16%). Adverse reactions, incidence of clinical wound infection and the healing time were analyzed.

Results: In 25 patients (75.7%) the wounds healed completely within 3 weeks. On the other hand, small ulcers remained in 3 patients (9.1%) whose burns were mainly deep partial. Three patients dropped out due to adverse reactions. One patient clinically showed signs of a wound infection during treatment.

Conclusions: Carboxymethylcellulose is a safe, and suitable material for treatment of partial thickness burns.

THE COMBINATION EFFECTS OF HYDROGEL DRESSING (VIEWGEL[®]) AND POLYURETHANE FILM DRESSING (CATHEREEP[®]) ON SPLIT-THICKNESS SKIN DONOR SITES

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Aim: Viewgel[®] is the new type of hydrogel dressing. It has no adhesiveness in itself, so it needs some methods of fixation. We fixed Viewgel[®] by the polyurethane transparent film dressing (Cathereep[®]), and examined the combination effects of them.

Methods: Viewgel[®] and Cathereep[®] were applied on 26 split-thickness skin donor sites, and had been observed for up to 2 weeks. The efficacy was evaluated by the 3 viewpoints of pain, infection and epithelialization. The usability was evaluated by the 3 viewpoints of sense of use, absorption and adhesion. The overall usefulness was judged from efficacy, usability, and safety.

Results: 22 cases were epithelialized completely in less than 2 weeks. 4 cases were suspended because of infection (2 cases), pain (1 case), maceration (1 case). We evaluated the efficacy as "excellent" in 88% of cases, usability as "excellent" in 88%, overall usefulness as "excellent" in 88%.

Conclusions: The combination of hydrogel dressing and polyurethane film dressing is very useful and safe in the treatment for split-thickness skin donor sites.

NOVEL CLINICAL POSTOPERATIVE RADIATION PROTOCOL FOR KELOID AND HYPERTROPHIC SCARS

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Aim: Before 2002, keloids and hypertrophic scars were uniformly treated with 15 Gy postoperative irradiation (old protocol) at our facility. Analysis of the therapeutic outcomes of patients treated with the old protocol showed that the recurrence rates of keloids and hypertrophic scars in the anterior chest wall and the scapular and suprapubic regions were statistically higher, while the recurrence rates in ear lobes were lower than other sites. Thus, we customized doses for various sites.

Methods: Between January 2002 and September 2003, 44 patients with 55 keloid sites were treated with surgical excision followed by the new protocol: electron-beam irradiation at a total dose of 10 Gy, 15 Gy and 20 Gy, varied by site. The recurrence rate and toxicity observed following this novel protocol were historically compared between 218 patients with 249 keloids treated with the old protocol of surgical removal followed by 15 Gy irradiation (without variation by site). A minimal follow-up time was 18 months. The statistical analysis was performed using Fisher's exact probability test.

Results: Total recurrence rate was 29.3% and 14.5% before 2002 and after 2003, respectively. Recurrence rate was reduced statistically in the anterior chest wall. In the ear lobe, there was no difference between the 15 Gy and 10 Gy groups.

Conclusions: Keloids should be treated with customized dose protocols, by site. The results suggest that keloid sites with a high risk of recurrence should be treated with 20 Gy/4 fractions/4 days.

GENERAL V; CASE REPORT

TREATING PSEUDOARTHROSIS WITH PLATELET RICH PLASMA

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Aim: Platelet Rich Plasma (PRP) has attracted attention as a safe and cost-effective source of growth factors that stimulate cells to regenerate tissue. Successful clinical applications of PRP have been reported. Pseudoarthrosis following bone fracture or osteotomy often requires a lot of efforts to treat. Two cases of pseudoarthrosis were successfully treated with PRP.

(Case 1) A nine-year-old girl with polydactyly underwent excision of redundant finger and osteotomy to correct alignment of proximal phalanx of thumb. Non-union was observed at the osteotomized phalanx. The non-union site was surgically refreshed and external fixation device was applied. PRP, obtained from the patient's peripheral blood was applied to the site. Bony union was observed after 4 weeks.

(Case 2) A twelve-year-old girl with Poland syndrome underwent distraction osteogenesis of her index finger. She broke the phalanx after the fixation device was taken off. The site developed into pseudoarthrosis. The non-union site was surgically refreshed and external fixation device was applied. PRP, obtained from the patient's bone marrow aspirate was applied with particles of cancerous bone taken from iliac diploe. Bony union was observed after 6 weeks fixation.

Discussion: PRP is a promising source of growth factors. We have previously confirmed that PRP processed from bone marrow aspirate contains the same high level of growth factors as that processed from peripheral blood. It also contains condensed marrow stromal cells. Great roles can be expected to play by PRP for regenerative medicine.

ORBITAL FLOOR RECONSTRUCTION WITH BIODEGRADABLE POLY(LA-CL,50:50) POLYMER IMPLANTS

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Aim: The purpose of this study was to share our clinical experience on the use of biodegradable poly (LA-CL,50:50) polymer implants to repair, large (>4 cm²), inferior orbital wall defects and to evaluate whether biodegradable poly(LA-CL,50:50) polymer implants adequately supports the orbital soft tissue contents.

Methods: Three patients who suffered orbital fractures, with >4 cm² bony defects in the inferior orbital wall, took part in the study. The inferior orbital wall was explored via subconjunctival approach. After repositioning of orbital content, each inferior orbital wall was reconstructed using a biodegradable polymer. Computed tomography and magnetic resonance imaging coronal sections were undertaken before the operation and 6 months postoperatively.

Results: The magnetic resonance imaging studies showed no abnormal tissue foreign body reactions in orbital region. The material showed adequate strength bone segments during the critical period of the bone healing. The bone healing seems to take place along the bone fragments.

Conclusions: Biodegradable poly(LA-CL,50:50) polymer are safe and reliable for the repair of the large defects (>4 cm²) in the inferior orbital wall. It seems that this is the first reported biodegradable material to promote bone healing along the bone fragments of the inferior orbital wall.

THE TREATMENT OF SKIN COLOR MISMATCH USING AUTOLOGOUS SKIN CELLS HARVESTING DEVICE

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The treatment of skin color mismatch such as hypopigmentation and post skin graft is not established yet. We experienced 2 patients of skin color mismatch treated with autologous skin cells harvesting device (ReCell[®], C3, Cambridge, UK). One was a patient of nevus Ota which had been treated with dry ice and caused hypopigmentation. Another one was a patient of burn contracture of the neck which had been treated with skin graft and caused skin color mismatch. We harvested 2 cm² of thin split thickness skin (0.2–0.3 mm) from surrounding area and made cell solution using the device. The color mismatch lesion was abraded to superficial layer of dermis and was applied the cell solution which include melanocytes. The wound was covered with a hydrophilic polyurethane dressing material (HydroSite[®], Smith & Nephew, London, UK). The dressing was opened 1 week after the surgery. Neither an antiseptic nor an antibiotic ointment was used and the graft was kept shaded after the surgery. The change of color was evaluated pre and post operatively with photograph and skin color measuring instrument (Dermaspectrometer[®], Cortex Technology, Hadsund, Denmark). In both patients epithelialization was complete within 10 days after the surgery. Although the postoperative inflammatory response is still remaining, favorable recovery of skin color is obtained on the 4th postoperative month. This method doesn't need complicated technique and doesn't take time to perform the operation, moreover it is low invasive to the donor site. Although we need more follow up time, it might be useful method for the treatment of skin color mismatch.

EFFICACY OF A NEGATIVE PRESSURE DRESSING FOR INTRACTABLE ULCER WITH POCKET FORMATION IN AN OUTPATIENT: A CASE REPORT

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Introduction: The Negative Pressure Dressing technique, which controls inhibitory factors in healthy wound healing, is an effective therapeutic modality dealing with intractable ulcer associated with pocket formation. We present a case of postoperative intractable ulcer associated with pocket formation located in the irradiated area. Using a negative pressure dressing, the patient was successfully treated as an outpatient after short-term admission.

Case: A 60-year-old male with laryngeal cancer developed an open wound with a pocket at the posterior neck after receiving treatments including surgery (laryngectomy and neck dissection) and radiotherapy (total dose of 50 Gy to the neck lymph nodes). In spite of conservative therapy such as the application of disinfectant and gauze compression over a two-month period, the ulcer became worse. Adverse effects prevented ulcer healing. He was admitted to our hospital to treat the ulcer with a negative pressure dressing. After receiving instructions from nurses in the management of the modality, he subsequently continued the therapy over a two-month period as an outpatient with a favorable outcome.

Summary: This case shows that under adequate instruction in the management of a negative pressure dressing this technique is easy to perform and can be useful in cases of outpatients suffering from intractable ulcer with pocket formation.

A CASE REPORT OF A PATIENT WITH DIABETIC GANGRENE OF THE LOWER LIMB WITH NECROSIS OF ALL THE MUSCLES ON THE ANTERIOR SIDE OF THE TIBIA WHO WAS SAVED FROM AMPUTATION

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A 64-year-old man came to our hospital complaining the pain of his right lower limb and difficulty in walking with a history of diabetes mellitus for about 20 years without management of the disease. He had recognized that his right ankle had been swollen for several months but he did nothing for it. He came to feel so severe pain in his right lower limb that he decided to come to our hospital. On the first examination, there was expansive swelling and severe redness of his right leg and foot with necrotic areas of the skin of the leg. The laboratory data showed severe inflammation and a bad condition of diabetes mellitus. X-ray films showed no lucent areas in the bone of his lower limb and gas in the soft tissue. He underwent an operation for debridement and drainage of his right leg and foot. The operation revealed that the muscles and fat tissue on the tibia were necrotic and melted and there were many abscesses along the tendons on the tibia. After the removal of the necrotic tissue and the infected tendons, the anterior surface of the tibia was exposed. After he underwent the management of diabetes mellitus, local treatment of the right lower limb, antibiotics therapy and grafting skin, his lower limb was cured and he came to be able to walk with equipment. He was saved from amputation of the lower limb in spite of the extensive gangrene of the leg and foot.

After all he came to our hospital because he had difficulty in walking. Body temperature was 38.1°C, the level of the blood sugar was 488 mg/dl, the level of HbA1c was 11.7%, the level of WBC was 29800/μl, the level of CRP was 30.3 mg/dl.

He underwent debridement and drainage of purulent exudation.

GENERAL VI; GROWTH FACTOR · ADHESION MOLECULE · CYTOKINES 1

HISTOLOGICAL EXAMINATION OF THE THIRD DEGREE BURNS APPLIED THE ARTIFICIAL DERMIS (AD) WITH TOPICAL USE OF BASIC FIBROBLAST GROWTH FACTOR (bFGF)

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Aim: To evaluate histological changes of the third degree burn wound simultaneously applied the AD (TERUDRMIS™), which works as a scaffold of dermis-like formation and topical use of bFGF that accelerates wound healing.

Methods: Two cases of severe burns were examined in half side test, after an informed consent. After full-thickness wound excision of third degree burns, the AD, of which bFGF had been topically sprayed over a collagen layer, was applied. Thereafter, bFGF was sprayed on the AD (FGF site) in every two days for two weeks, and mesh split-thickness skin graft was applied. Histological examination was done by H-E stain, Elastin stain, and several immunohistological stains (collagen IV, TGF- β typeII receptor, CD34).

Results: Vigorous neovascularities were detected in the neodermis early in FGF site, and epithelization of the graft was accelerated compared to the control site. Cicatricial findings were also excellent at FGF site.

Conclusions: Simultaneous use of the AD and topical bFGF may promote the formation of neovascularities in the dermis-like tissue and accelerate epithelization of the mesh-grafted wound.

TEMPORAL IMMUNOHISTOCHEMICAL LOCALIZATION OF A VARIETY OF GROWTH FACTORS IN HORIZONTAL SECTIONS OF MURINE CUTANEOUS WOUNDS

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Aim: The temporal immunohistochemical localization of platelet-derived growth factor A (PDGFA), basic fibroblast growth factor (bFGF), FGF receptor-1 (FGF-R1), FGF receptor-2 (FGF-R2), epidermal growth factor (EGF), keratinocyte growth factor (KGF), transforming growth factor β 1 (TGF- β 1), and transforming growth factor β 2 (TGF- β 2) were examined during wound healing in murine skin. In addition to them, α -smooth muscle actin (α -SMA), which was reported to be transiently expressed by myofibroblasts during experimental wound healing, was also evaluated.

Methods: Frozen horizontal sections taken from the full-thickness skin excisional wound were reacted with the appropriate antibodies using avidin-biotin-peroxidase complex method. For microscopically analyzing the stainings of α -SMA and these growth factors, granulation tissues (at days 5 to 10) or scar tissues (at days 14 to 20) were evaluated for the horizontal sections of wounds. The number of positive and the total number of fibroblasts were counted.

Results: More than 80% of fibroblasts were α -SMA positive during 7 to 10 days after wounding, and at later time points the ratio of positive cells decreased. At day 5, more than 80% of the fibroblasts were bFGF and PDGFA positive, and afterward the number of positive cells decreased gradually. The ratio of FGF-R1, FGF-R2 and EGF positive cells increased and peaked at day 10, and then decreased. The ratio of TGF- β 1, TGF- β 2 and KGF positive cells increased throughout day 5 to 20.

Conclusions: These findings suggest that expression of growth factors during the excisional skin wound healing showed three stages.

EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN THE COURSE OF OSTEOINDUCTION BY BONE MORPHOGENIC PROTEIN-2

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When recombinant human bone morphogenetic protein-2 (rhBMP-2) is implanted in soft tissues, bony tissue is induced during the course of endochondral ossification. The relationship between endochondral ossification and vascularization is important in bone formation, and vascular endothelial growth factor (VEGF) is considered to play an important role in this process.

In this study, the immunohistological localization of VEGF was investigated in rhBMP-2-induced ectopic endochondral ossification in the calf muscle of the rats. In addition, the characteristics of anti-VEGF antibody-reactive cells were histologically investigated using electron microscopy to examine the cause of endochondral ossification induced by recombinant human bone morphogenetic protein-2. The role of VEGF in rhBMP-2-induced osteoinduction and vascular induction was studied by observing the relationship between the localizations of anti-VEGF antibody-reactive cells and vascularization. During the process of rhBMP-2-induced ectopic endochondral ossification, fibroblast-like cells, which were located at the margin of the implant and reactive to BMP-2 at 5 days, were positive for VEGF immunostaining. Hypertrophic chondrocytes appeared 9 days and osteoblasts appeared 14 days after implantation, and all these cells were reactive with anti-VEGF antibody. Bony trabeculae subsequently appeared in the muscle, and new blood vessels were formed besides the trabeculae. These findings suggested that rhBMP-2 induced the differentiation of undifferentiated mesenchymal cells to chondrocytes and osteoblasts, and these differentiated cells expressed VEGF, creating an advantageous environment for vascularization in bony tissue.

ANALYSIS OF INTRACELULAR SIGNAL TRANSDUCTION OF BASIC FIBROBLAST GROWTH FACTOR-STIMULATED FIBROBLAST-COLLAGEN MATRIX CONTRACTION

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Fibroblast-collagen matrix contraction has been used as a model system to study how cells organize connective tissue. Previous work showed that lysophosphatidic acid (LPA)-stimulated floating collagen matrix contraction is independent of Rho kinase while platelet-derived growth factor (PDGF)-stimulated contraction is Rho kinase-dependent. The current studies were carried out to determine the signaling mechanisms of basic fibroblast growth factor (bFGF)-stimulated fibroblast-collagen matrix contraction. LPA, PDGF and bFGF each equally well stimulated collagen matrix contraction. Both of two kinase inhibitors, LY294002 for phosphatidylinositol-3-Kinase (PI3K) and Y27632 for Rho kinase, suppressed the bFGF-stimulated fibroblast-collagen matrix contraction. With bFGF stimulation, fibroblasts in collagen matrix spread with prominent stress fiber network formation. The present study implicates PI3K \rightarrow Rac \rightarrow Rho \rightarrow Rho kinase as being involved in bFGF-stimulated collagen matrix contraction. Since this pathway is similar to PDGF but not LPA, the combination therapy with bFGF and LPA is worthwhile to future strategy for the treatment of chronic cutaneous wound. The elucidation of bFGF-triggered signal transduction may be an important clue to understand the roles of bFGF and study the possibility of the combination therapy in wound healing.

GENERAL VII; GROWTH FACTOR · ADHESION MOLECULE · CYTOKINES 2

BASIC FIBROBLAST GROWTH FACTOR STIMULATES HUMAN KERATINOCYTE MOTILITY THROUGH HUMAN FIBROBLAST

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Topical application of human recombinant basic fibroblast growth factor (bFGF) promotes wound healing including reepithelialization. bFGF, however, has been reported to have little *in vitro* effects on keratinocyte compared to human dermal fibroblasts. In this study, we clarify the mechanisms of bFGF-stimulated keratinocyte migration at least in a part. Normal human keratinocytes, seeded on coverslips which were non-coated 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 suggests 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, probably by $\alpha 2\beta 1$ integrin.

EXAMINATION OF $\alpha v\beta 6$ INTEGRIN AND Ki-67 FOR BURN AND PRESSURE ULCER EPIDERMAL CELLS

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Objective: Migrating epidermal cells up-regulate the expression of integrins, such as $\alpha 5\beta 1$, $\alpha v\beta 5$ and $\alpha v\beta 6$ through the reepithelialization process of acute wounds. To date, there have been no reports on the role of integrin $\alpha v\beta 6$ in burns and pressure ulcers. In this study, epidermal cell adhesion molecule expression of $\alpha v\beta 6$ and migrating epidermal cell localization were researched. The proliferation period of cell localization was examined using the Ki-67 antibody and migrating epidermal cells were observed indirectly. Laminin (LN) as an indicator of reepithelialization and its correlation were examined.

Method: Specimens from 7 burn cases and 16 pressure ulcer cases were used for materials. Immediately after removal of an ulcer bearing portion of the skin, a frozen specimen was made and two-layer staining using the immunohistochemical method, the ABC method and the indirect method was carried out. The ABC method was used for $\alpha v\beta 6$ and Ki-67 and the indirect method was used for LN. Hematoxylin-eosin staining was performed for each preparation.

Results: $\alpha v\beta 6$ and LN Staining Findings: Expression of $\alpha v\beta 6$ was observed in the apical region of the epidermal cells in all burn cases and in 12 pressure ulcer cases. There was a correlation between $\alpha v\beta 6$ and LN in both the burn and pressure ulcer groups. Ki-67 and LN staining Findings: The apical region of Ki-67 negative epidermal cells in all the burn cases and in seven pressure ulcer cases. There was a correlation between Ki-67 and LN in both groups. Histological Examination: Significant differences were recognized both groups. **Conclusion:** Expression of $\alpha v\beta 6$ in the positive part of the apical region of the epidermis may be used as an active indicator of epidermal migration, and expression of Ki-67 in the negative part may contribute to a better understanding of the progression of epidermal migration.

GENERAL VIII; INFECTION OR INTRACTABLE ULCERS/ DECUBITUS

SURGICAL SITE INFECTION (SSI) AMONG 1,509 PATIENTS IN THE LAST 3 YEARS

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Aim: The purpose of this study is to assess the background data of SSI in the last 3 years, from April of 2002 to March of 2005.

Methods: The SSI surveillance was started from April of 2003 in our department. The retrospective data from April of 2002 to March of 2003, Season 1, and the prospective data of each consecutive year, Season 2 and 3, were enrolled in this study. The incidence of SSI of each Season was evaluated, together with regard to the contamination level of the operations. The correlation between the SSI and antibiotic prophylaxis was also analyzed.

Results: This study included 425 patients in Season 1, 409 in Season 2 and 497 in Season 3, and the incidence of SSI was 14.8%, 10.3% and 10.1%, respectively. In the severe contaminated operations, the incidence of SSI of Season 1 was high, 57%, compared with 33% in the combined data of both Season 2 and 3. The prophylactic antibiotics were administered until 3 post-operative day (median value) in Season 1 and 2 postoperative day in Season 2 and 3 ($p < 0.0001$).

Conclusion: The SSI surveillance applied from April of 2003 decreased the incidence of SSI, especially in severe contaminated operations. During this period, the use of prophylactic antibiotics was significantly decreased compared with the period before the surveillance. The infection management during operation is essential for SSI control.

THE EFFECT OF CANDIDA ALBICANS INFECTION ON CYSTEAMINE-INDUCED DUODENAL ULCER IN RATS

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Background and Purpose: It was known widely that *Candida albicans* not only inhabits frequently the gastrointestinal tract of humans, but also was cultured in the ascites in the patients who have undergone the operation for gastro-duodenal ulcer perforation. This experiment was performed to clarify the effect of *C. albicans* infection on the gastroduodenal ulcer in rats.

Methods: The patients with gastroduodenal ulcer perforation from 1993 to August 2004 were discussed. In this study, male Wistar rats (220–250 g) were separated into the *Candida* group and the control group. After the rats were anesthetized with ether, the rats were administered with cysteamine (310 mg/kg body weight) three times intragastrically every 4 hours; the *Candida* group rats were administered with *C. albicans* at one hour before and 12, 24 hours after the first dose of cysteamine. The control group rats were administered with the saline. The rats were sacrificed after 72 hours after the first administration of cysteamine. The depth of ulcer, the ulcer area and the incidence of ulcerate perforation in *Candida* group rats were compared to that of the control group. The immunohistochemical stain was done with an anti-Sap2 (secretory aspartyl protease 2) monoclonal antibody.

Result: In ascites culture, the incidence of *Candida* infection was 38% of all cases with gastroduodenal ulcer perforation. In the pathological examination, the incidence of *Candida* infection was 44% of the cases. In our experiment, the rate of duodenal ulcer perforation in the *Candida* group is 94.1% (16/17) that was higher than the control group (26.7%, 4/15). The size of duodenal ulcer area in the *Candida* group was larger than the control group; the survival rate of the *Candida* group was significantly lower than that of the control group. In the pathological analysis, not only *Candida* was observed in the ulcer bed, but also the filtration of eosinophils was seen in the ulcer bed by Luna stain in the *Candida* group. In immunohistochemical analysis, the ulcer bed in the *Candida* group was positive, whereas the control group was negative.

Conclusion: It was shown that the infection of *C. albicans* promotes the process of duodenal ulcer perforation in cysteamine-induced ulcer in rats.

EXAMINATION OF DIABETIC FOOT NECROSIS TREATED WITH MAGGOT DEBRIDEMENT THERAPY AND SPLIT THICKNESS-SKIN GRAFT

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Aim: We announced that we examined diabetic necrosis foot treated with maggot. We got good result.

Methods: We recognized that maggot is effective to wound repair from any thousand years ago. Maggots grown up flies one week. We changed maggot two times per a week. We judged operation at each wound dressing change. This time patient's had many pus at raw surface, we performed split thickness-skin graft from inguinal skin. Conclusively patient preserved lower leg.

Result and Conclusion: We used sterilized maggots. Maggots ate necrotic tissue and wound bed prepared and promoted granulation tissue. We got good result adding to split thickness-skin graft to reduction raw surface. We reported a few Maggot Debridement Therapy in Japan.

EFFECTIVENESS OF PERIPHERAL VASCULAR ARTERY RECONSTRUCTION TO PATIENTS WITH ISCHEMIC ULCER AND GANGRENE

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We examined for effectiveness of arterial reconstruction to patients with ischemic ulcer or gangrene. Between January 2000 and June 2005, 24 patients (26 legs) with arteriosclerosis obliterans of Fontaine underwent arterial reconstruction. 9 patients had ulcer and 17 patients had gangrene. In those, 5 patients had gangrene beyond metatarsal bones. Local infection of ulcer or gangrene was recognized in 12 patients by MRSA or P. aeruginosa. After operation, the average ankle brachial pressure index increased 0.32 to 0.73 and transcutaneous oxygen tension increased 11 mmHg to 38.7 mmHg, both of which had significant difference. The primary patency was 79.8% in one year, 70.9% in two years. 21 legs of 20 patients got wound healing and their ABI increased to more than 0.5 after operation. It was difficult to control infection in 4 cases with local infection beyond metatarsal bones and 3 legs needed major amputation and one case died of chronic respiratory failure even though they had no graft failure. Limb salvage rate was 86.9% in one year and 74.5% in two years.

Conclusion: Ischemic ulcer or gangrene healed by arterial reconstruction in most cases, but when the case had local infection beyond metatarsal bones, it was difficult to control infection and major amputation should be performed in spite of patent graft.

GENERAL IX; GENE THERAPY · MISCELLANEOUS 1

POSITIVE EFFECT BY TAKING DOWN FOOT FOR WOUND HEALING OF CRITICAL LIMB ISCHEMIA (CLI)

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Aim: In the case of CLI, putting up foot with ulcer causes an ischemic pain, while taking down foot decreases it. We hypothesized that to take down foot improves the circulation of CLI. To evaluate an increase of blood flow to ischemia limb by taking down foot, we compared skin perfusion pressure (SPP) values of putting up foot position with that of taking down foot position. SPP can accurately show microcirculation in the subcutaneous tissue, and predict wound healing. We utilize SPP data for a diagnosis of CLI.

Methods: We measured SPP values of the lower extremities of 20 volunteer without PAD.

Results: In the normal legs, the average SPP value of putting up foot position is 80 mmHg, while that of taking down foot position is 120 mmHg. SPP data of taking down foot is significantly higher compared with that of putting up foot. (P < 0.0001)

Conclusions: In the case of CLI, to take down foot can improve the circulation of CLI and is useful for ischemic pain control.

GENERAL X; GENE THERAPY · MISCELLANEOUS 2

ROLE OF IL-6 IN THE DEVELOPMENT OF KELOID SCAR

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Aim: Keloid scar is the result of an abnormal wound healing. In this study we evaluated the role of inflammatory cytokine, IL-6, in the pathogenesis of keloid scar.

Methods: Primary cultures of keloid fibroblasts and adjacent normal dermal fibroblasts from three patients were studied. The amount of IL-6 secretion and the mRNA levels of IL-6 and its specific receptor IL-6R-alpha as well as gp130 (IL-6 signal transducer protein) and some other downstream targets in the IL-6 signaling were measured.

Results: Our results showed that the level of IL-6 secretion was higher in keloid fibroblasts compared to normal fibroblasts. Stimulation of normal fibroblasts by IL-6 peptide increased mRNA levels of collagen type I alpha and fibronectin. The mRNA levels of IL-6R-alpha, gp130, and some other downstream targets of IL-6 signaling such as STAT3 and ELK1 genes were decreased.

Conclusions: Our results suggested that IL-6 pathway may be involved in keloid scar formation. Thus, we suggest IL-6 blocking methods as a potential therapy for keloid scars.

A SKIN ULCER HEALING MODEL OF CULTURED SKIN

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Aim: Experimental comparisons of skin ulcer healing are difficult. Using a bilayered human cultured skin model, we made a skin ulcer model and basic fibroblast growth factor (FGF) was compared with control.

Methods: We made partial defect of epidermis in the three-dimensional cultured skin model by CO₂ LASER. The Skin ulcer model was cultured with FGF during one week. Their healing were compared with control histologically (HE stain).

Results: The all FGF skin ulcer model had thin epidermis on the surface of ulcer at four days. Although some control model had no epidermis on it.

Conclusions: The development of three-dimensional cultured human skin models has resulted in their use for testing the skin irritancy of cosmetics and other materials as an alternative to animal experiments, which are increasingly becoming controversial.

We speculate that since cultured skin models are not supplied by blood flow, they can test the materials that repair the wounds directly.

THE EVALUATION OF WOUND REPAIR AT RESECTION OF RAT GASTRIC MUCOSA AND EFFECT OF PHOTOCROSSLINKABLE CHITOSAN

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Aim: There are many reports about acceleration for wound healing of chitosan hydrogel; we studied whether photocrosslinkable chitosan promoted wound healing of ulcer after mucosal resection.

Methods: The chitosan hydrogel, sodium hyaluronate, and hypertonic saline (each 0.5 ml) were administered into gastric submucosal layer of rats after anesthesia. We measured the area of artificial ulcer (1, 2, 4, 7 days), and performed the pathological examination (1, 2, 4, 7 days).

Results: The areas of artificial ulcer 7 days after mucosal resection were $5.6 \pm 1.3 \text{ mm}^2$ in the chitosan hydrogel with ultraviolet irradiation group, $7.3 \pm 2.1 \text{ mm}^2$ in the sodium hyaluronate-treated group, $8.5 \pm 2.4 \text{ mm}^2$ in the hypertonic saline-treated group.

Conclusions: Wound healing using photocrosslinkable chitosan was better than that of using hypertonic saline and sodium hyaluronate.

SYMPOSIUM 1: ANGIOGENESIS AND WOUND HEALING (BASICS AND CLINICS)

HARMFUL INFLUENCE OF POVIDONE-IODINE DISINFECTANT AND PROMOTIVE EFFECT OF TAP WATER RINSING ON WOUND HEALING. EXPERIMENTAL STUDIES USING HAIRLESS MICE

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Aim: It has been accepted that antiseptics induces delay in wound healing. We evaluated harmful influence of the disinfectant on suppression of wound healing using mice experimental model.

Methods: Full thickness skin-defective wound, measuring $2 \times 2 \text{ cm}$, was made on the back of HR-1 hairless mice. A total of 36 mice were divided into four groups, in which the following rinsing solutions were employed: (1) tap water, (2) isotonic saline, (3) 10% povidone iodine (PVP-I), and (4) 10% PVP-I followed by saline 15 to 20 minutes later. After rinsing, wound was covered with food wrap film dressing to keep moist environment. The period until wound healing were evaluated.

Results: The period until suppression of wound healing was 14.6 ± 0.7 days in group 1, 20.0 ± 1.6 days in group 2, 24.1 ± 1.8 days in group 3, and 17.3 ± 2.2 days in group 4. Wound healing was significantly delayed by 10% PVP-I followed by saline compared with the rinse with tap water, saline and 10% PVP-I followed by saline ($p < 0.001$). The experiment clearly proved the suppression of wound healing by the PVP-I rinse.

Conclusions: It is worthy of further evaluation that the tap water rinse was more effective than the saline rinse.

INHIBITION OF WOUND CONTRACTION BY BASIC FIBROBLAST GROWTH FACTOR IMMERSSED IN SPONGY COLLAGEN MATRICES

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To assess the role of bFGF in wound contraction, the present study compared α -SMA expression and degrees of wound contraction after applying collagen sponge with or without bFGF to open wounds in rats. When compared with controls, the wounds treated with matrices containing bFGF alone showed significant inhibition of wound contraction by day 42, and decreased expression of α -SMA by day 14. Analysis of TUNEL indices showed a peak on day 14, with peak indices being significantly higher in the wounds treated with sponges containing bFGF alone than in the other groups. Double staining for TUNEL and Hoechst showed that some myofibroblasts had undergone apoptosis. These results suggest that bFGF treatment downregulates α -SMA expression, along with an increase in apoptosis of myofibroblasts, resulting in the inhibition of wound contraction in open wounds.

ACCELERATION OF WOUND ANGIOGENESIS IN CASES OF ARTIFICIAL DERMIS GRAFTING

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Aim: Currently, to treat skin defects with artificial dermis (AD), two surgical procedures where the AD grafting and another secondary skin grafting are required. The purpose of this study was to achieve simultaneous grafting of the AD and the split-skin by enhancing the wound angiogenesis.

Methods: I experiments 1) Full-thickness wounds created on the back of the rats were treated with the AD (Terudermis[®], TERUMO Co., Japan). 2) Simultaneous grafting of the AD and skin was performed using the same rat model. The AD were treated with PDWHF (Platelet Derived Wound Healing Factors), cultured endothelial cells and fibroblasts. II. Clinical trial: In a case of 49% TBSA burn, full-thickness burned wound on his leg was treated by this method. The AD was treated with autogeneous PDWHF together with allogenic cryopreserved cultured endothelial cells and fibroblasts.

Results: I. 1) When the AD were treated with PDWHF combined with cultured cells, vascular invasion into the AD was obtained 5 days after the surgery. 2) The skin grafted immediately after the AD grafting was completely taken. II. In the clinical case, the grafted skin was completely taken and satisfactory tissue texture was obtained.

Conclusions: The present study revealed that treatment with PDWHF, combined with cultured endothelial cells and fibroblasts, accelerated wound angiogenesis. By this method, immediate skin grafting following the AD grafting is possible.

SYMPOSIUM 2: WOUND DRESSING MATERIALS

EVALUATION OF THE DEGRADABILITY OF CHITOSAN HYDROGEL

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Chitosan hydrogel is well known as a wound dressing and tissue adhesive material showing biocompatibility, anti-infective activity and the ability to accelerate wound healing. Our previous report showed that medium(DMEM/F12)-containing photocrosslinkable chitosan hydrogel (medium-Az-CH-LA) easily degraded and promote wound epithelization.

The degradation effect of human myeloid leukemia HL-60²⁰ cells or lysozyme on chitosan hydrogel-PBS (PBS-Az-CH-LA) and medium-Az-CH-LA was determined using in vitro assay.

Microscopic findings revealed that HL-60 cells degraded the medium-Az-CH-LA, but hardly degraded PBS-Az-CH-LA. Although the degradation ratio of the medium-Az-CH-LA was found higher than the PBS-Az-CH-LA, no significant difference in degradation ratio by lysozyme was observed.

These findings suggested that direct cell-chitosan hydrogel interaction may be correlated with the enhancement of the degradation activity upon addition of medium (DMEM/F12). Another study showed that chitosan induces apoptosis of peripheral macrophages by activating the mannose receptor, while addition of excess sugars, such as mannose, inhibit apoptosis. Along these findings, the addition of the medium (DMEM/F12) may inhibit apoptosis of infiltrating cells and this way promote the degradation of the chitosan hydrogel by these infiltrated cells.

CURRENT WOUND MANAGEMENT IN OUR ER –WOUND CLEANING SYSTEM AND MOIST DRESSING

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Introduction: Wounds should be managed cleanly and taken careful consideration both for better cosmetic and functional outcomes even in ER. The important elements of wound management must be how to clean for prevention of wound infection and how to close for better granulation.

Methods: Wound cleaning and debridement system JETOX[™] (TavTech LTD., Israel) is originally used for cleaning and debridement of venous ulcers or burns with accelerated saline by compressed oxygen. We use this system for dirty scratch, cut or contused wounds in ER. After cleaning these wounds, custom-made wound care, called 'moist dressing', is followed in ER with some dressing devices to keep moist environment for better wound healing.

Results: As virtually painless, all wounds can be washed without local anesthesia, potential allergen. Less saline is needed for cleaning and removing dirty foreign body or non-viable tissue because of adjustable jet even for cavity wounds with cotton tips. No patients showed infection of washed wounds so far, even without local disinfection. Moist dressing reduces the frequency of attending hospital.

Conclusion: This cleaning device is useful for wound management in ER. Keeping moist environment with dressing devices followed by cleaning using this device leads better wound healing.

PROBLEMS IN DIFFUSING WOUND DRESSINGS AND FUTURE ISSUES

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Wound dressings have been broadly used for treatment of apellous wounds like burns and pressure ulcers, having effect to promote wound healing. Recently various materials and forms of wound dressings have been developed for earlier and more aesthetic wound healing. On the other hand, many doctors and nurses are confused, "When which wound dressing should be used for which wound?" The followings are problems of wound dressings;

1. Medical reimbursement: Medical reimbursement is not applied to all types of wounds and periods of treatment.
2. Cost-effectiveness: Wound dressings, which can receive reimbursement, are too expensive to use for wounds beyond coverage case of reimbursement.
3. Variety: It is confusing because of too many kinds of wound dressings.

In our hospital, the wound dressing is selected regarding to the wound site, the wound condition, the exudate condition, and frequency of hospital visit. It is important to think when, which one, how to use, for 'Moist Wound Healing'. Wound dressings contribute to reduce mental and physical burdens of patients and workloads of the medical staff, because of less exchange with using wound dressings. Thus, it also reduces total medical cost.

Future issues are more diffusion with improving some functions like moisture retention and adherence, larger application of medical reimbursement, and establishment of the appropriate usage method.

EFFICACY OF SKIN PROTECTIVE POWDER AS DRESSING MATERIALS

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Objective: This study sets out to evaluate the skin protective powder for the three difficult situations for conventional dressing materials in basic and clinical research.

Patients and Methods: In basic research, we evaluated and compared the wound healing: formation of granulation tissue by the total skin defect using db/db mice model between powder and film dressing material. Clinically, twelve patients with ulcerations were treated with skin protective powder. Each of these cases was evaluated by process of wound healing.

Results: In basic research, the thickness of granulation tissue of Powder was 2.86 times thicker than film dressing material. Clinically, in eleven cases the wounds healed completely after application of powder. Only in one case, was it necessary to stop the powder treatment and change to another type of dressing material. This case involved a deep burn ulcer of face, which was very fragile and edematous, and the granulation tissue of neo-epithelialized skin was easy to peel off during changing of the dressing.

Conclusion: Skin protective powder was evaluated and indicated for the three types of ulceration:

1 Contacting ulcer: Contacting face-to-face walls each other, known as kissing ulcer.

2 Ulcer and erosion of complex contour and morphology.

3 Ulcer and erosion exposed with contamination of massive liquid stool and vaginal discharge.

SYMPOSIUM 3 -1: STRATEGIES FOR TREATMENTS OF THE INTRACTABLE ULCERS 1

TOPICAL TREATMENT FOR SEVERE ULCERATIVE COLITIS — BASIC STUDIES

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Aim: To improve QOL of the patients with severe ulcerative colitis, we devised a drug delivery system that would repair the colonic lesion, and basically examined the efficacy of the novel material

Methods: We dissolved 70% deacetylated chitin(DAC-70) and 5-aminosalicylic acid(5-ASA) in a HCl solution to prepare the device. The adhesiveness between the devised agent and human colonic mucosa was measured by our own method. Release profile of the 5-ASA was studied in vitro. Therapeutic effects of the device were examined using rat colitis models induced by an acetic acid solution. The devised agent was intracolonicly applied for 7 days from the next day of the colitis-induction.

Results: The device was viscoelastic solution with pH range 6.6–6.8. The adhesiveness of the solution was stronger than that of hyaluronic acid solution clinically used. More than 90% of the 5-ASA in the device was slowly released for 6 hours at 37°C. The all animals applied the acetic acid solution contracted severe ulcerative colitis. Such injured sites were favorably repaired by the daily administration of our device: namely, six of 9 treated animals normally survived for longer than 4 weeks, while all 6 non-treated rats died within 5 days after the induction of the colitis. The necropsy revealed that every cause of the death was perforation of the colonic ulcer. Histopathology showed that the severely injured sites of the treated rats were favorably repaired by the daily administration of our device. In contrast, that of the non-treated animals exhibited significant transmural necrosis and degeneration of the colonic walls.

Conclusions: Topical application of “DAC-70+ASA solution” suggested to be useful to repair the injured sites of ulcerative colitis.

VACUUM ASSISTED SHOELACE TECHNIQUE (VAST) AND VACUUM ASSISTED NEOVASCULAR FORMATION (VANF); NEW TREATMENT OPTIONS FOR CHRONIC SKIN ULCERS AND NON-HEALING WOUNDS

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In clinical practice, complex chronic wounds are slow to heal and difficult to manage. The recently introduced technique of topical negative pressure therapy (vacuum-assisted closure; VAC) has been developed to try to overcome some of these difficulties. We used topical negative pressure therapy in combination with a shoelace technique (vacuum assisted shoelace technique; VAST) or artificial dermis replacement (vacuum assisted neovascular formation; VANF) for treating complex chronic wounds and wounds were successfully closed.

VAST: The aim of this technique is to reduce the size of ulcer and let it heal spontaneously. After debridement of the ulcer, sponges were placed into the cavity of the ulcer and elastic vessel loop was applied in a shoelace fashion to the skin edges, then covered with polyurethane film and applied negative pressure. Using this technique, the size of the ulcer was reduced significantly as well as the control of wound infection was achieved. **VANF:** When the wound was large and the wound bed was not adequate for skin grafting, VANF method was useful. In this method, artificial dermis was placed after the debridement of the ulcer, and then VAC system was applied over the artificial dermis. Using this method, neodermis formation was found to be adequately vascularized to accept thin split thickness skin graft within 7 to 10 days.

AVAILABILITY OF MEASUREMENT OF SKIN PERFUSION PRESSURE FOR CRITICAL ISCHEMIC LIMBS (CRITICAL LIMB ISCHEMIA)

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Purpose: In our hospital, the wound care center is established in January, 2002, and the incurable ulcer for which amputation under thigh is recommended at another hospital is treated. In the United States, the patient of amputation lower limbs due to incurable ulcer reaches up to about 60,000 cases, and the mortality rate is reported on 40% in the cases of major amputation above knee within two years after amputation.

On the other hand, success rate of bypass grafting (revascularization) is reported 85–90% and rate of limb salvage at 5 years is reported 81%. Therefore, we are treating critical ischemic limbs by the policy of enforcing limb salvage with bypass grafting (revascularization) as much as possible.

Objective and the Method: It is reported that 93% of chronic wound is cured with 30 mmHg of skin perfusion pressure(SPP), and recovers oppositely only by 18% in 20 mmHg of SPP. Therefore, we think that 30 mmHg or more SPP is necessary for wound healing (45 mmHg ideally). Then, we operated bypass grafting (revascularization), with angiography to the metatarsal artery. The measurement of SPP would be done to 18 cases (four dialysis patients) operated bypass grafting in two years from January 2003. After bypass grafting (revascularization), we measured SPP again and did surgical debridement to the cases whose SPP was improved.

Result: 15 of 18 cases became successful limb salvage, but 2 cases died after discharge from hospital. In addition, one case died with myocardial infarction and one case underwent major amputation.

Summary: The measurement of SPP was useful for the evaluation of blood circulation of critical limb ischemia and it also had clinical benefits in identification of the site of amputation and the site of peripheral anastomosis.

SYMPOSIUM 3 -2: STRATEGIES FOR TREATMENTS OF INTRACTABLE ULCERS 2

THERAPEUTIC STRATEGY FOR INTRACTABLE ISCHEMIC FOOT ULCERS

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Introduction: Severely intractable ischemic foot ulcers due to occlusive peripheral arterial diseases have been unreconstructable. Since the versatility of therapeutic angiogenesis using autologous bone marrow cell has been reported, these affected limbs have been able to be preserved in conjunction with several cell therapeutic approaches. In this paper, therapeutic algorithm for these ulcers was attempted to be established based on our clinical results.

Materials and Methods: 11 cases who have had severely intractable ischemic foot ulcers were reviewed. All cases were classified as Fontaine grade IV and underwent therapeutic angiogenesis using their autologous bone marrow cell implantation followed by minor surgical procedures including toe amputation, skin grafting, bone marrow-impregnated collagen sponge grafting and allogeneic cultured dermal substitutes.

Results: 9 patients successfully have their ulcers healed with less pain and good ambulating. However, 2 cases have eventually the affected limb amputated due to severe infection.

Conclusions: Since the incidence of occlusive peripheral arterial diseases has increased, such intractable foot ulcers can also be estimated to increase. Therefore, therapeutic algorithm that we proposed might be applicable for these diseases.

DIAGNOSTIC AND TREATMENT STRATEGY FOR CHRONIC VENOUS LEG ULCERATION

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Aim: To determine the indicative duplex-derived parameters reflecting the progression of chronic venous insufficiency, and to determine the treatment strategy for venous leg ulceration.

Methods: The distribution of venous insufficiency including the superficial, deep, and perforating vein insufficiency was determined by duplex ultrasound. The main duplex-derived parameters assessed were the diameter (cm), duration of reflux (s) and the peak reflux velocity (cm/s). Patients with superficial insufficiency were treated with stripping operation, these with deep vein insufficiency were treated with deep vein valvuloplasty, and these with perforator incompetence were treated with subfascial endoscopic perforator surgery.

Results: Among 74 limbs with venous ulcers, isolated superficial venous incompetence was found in 22 (30%), superficial combined with deep vein insufficiency in 13 (17%), superficial combined with perforating vein insufficiency in 12, and superficial combined with deep and perforating vein in 15 (19%) limbs. In superficial venous incompetence alone, the duration of reflux was significantly shorter in patients with ulceration than these without ($p = 0.02$). On the contrary, peak reflux velocity was significantly higher in patients with leg ulceration ($p < 0.0001$). Any statistically significant differences were found in duplex-derived parameters in patients who had deep or perforating vein insufficiency. Venous ulcer healed within 1.5 months after operation, but recurred in 2 patients who had chronic rheumatic arthritis and tricuspid regurgitation, respectively.

Conclusions: These data suggest that superficial venous insufficiency may play a major role in the disease development of ulceration. Duplex-derived duration of reflux are widely used for the evaluation of venous reflux, however, our data suggest that the peak reflux velocity is more important factor in the progression of chronic venous insufficiency.

SYMPOSIUM 4: SOMATIC STEM CELLS AND TISSUE REGENERATION

MODULATING THE INFLAMMATORY RESPONSE AT WOUND SITES TO IMPROVE THE QUALITY OF HEALING

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The process of adult tissue repair inevitably involves a robust inflammatory response, whereby neutrophils and macrophages are drawn to the site of tissue damage. In embryos where there is a much reduced inflammatory response, skin wound healing proceeds without subsequent scarring and we have recently shown that the same appears to be true of neonatal wounds in PU.1 null mice which are genetically incapable of raising an inflammatory response (1). A recent microarray comparison of wound-expressed genes in PU.1 null versus wild-type sibling mice has revealed several genes that appear to be a consequence of inflammation (2), and we have performed a temporal in situ hybridization study to characterize which cells are expressing these genes and at what stage in the repair process. We have begun to knock down some of these genes using the same Pluronic gel delivery of knockdown genes recently utilized to suppress connexin43 ($C \times 43$) at the wound site, and which reduced the inflammatory response and improved skin wound healing in both neonatal and adult mice (3). We hope this strategy of knocking down "inflammation-associated" genes at the wound site may reveal novel ways to improve the quality of healing in the clinic.

References:

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TISSUE REGENERATION BY ADIPOSE-DERIVED STEM CELLS; A MILESTONE FOR CLINICAL APPLICATION

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Introduction: Adipose-derived stem cells (ASCs) are promising for future cell therapy and tissue engineering as autologous adult stem cells. In this paper, we present our recent study using ASCs.

Materials and Methods: Experimental animals that we have used included Green Fluorescence Protein transgenic mice, rats, rabbits, dogs and pigs. ASCs were harvested from each animal according to the established protocol and cultured in control medium. Passage 2 to 3 ASCs were subsequently induced into a variety of cell lineages both *in vitro* and *in vivo*. All specimens were evaluated macroscopically, histologically and immunohistochemically.

Results: ASCs isolated from all animal races were successfully differentiated into adipogenic, chondrogenic and osteogenic lineages *in vitro*. In addition, mature tissue including fat, bone, cartilage, bone marrow, granulation tissue and periodontal tissue was regenerated in GFP mice and rats model *in vivo*.

Conclusions: ASCs were proven to be existed in a variety of animal races as well as human. These findings were considered to make an important role on developing a future clinical application in the field of wound healing and tissue engineering.

THE ROLE OF SHC-GENE DEFICIENT BONE MARROW-DERIVED STROMAL CELLS IN WOUND HEALING

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Aim: As the mesenchymal stem cells or stromal cells play important roles in cutaneous wound healing, further detailed relationship with neuronal expressions are tested in Shc-deficient mouse derived bone marrow derived stromal cells for clinical diabetic or other neuronal-associated impaired wound healing.

Methods: The human derived mesenchymal stem cells (hMSCs) and Shc-deficient mouse derived stromal cells were investigated for neuronal factor analyses and in vivo wound healing.

Results: The hMSCs expressed ShcA, ShcC, stathmin and SCG10 proteins. ShcC-deficient mouse derived bone marrow stromal cells were significantly lowered in proliferation in comparison with that of the litters. In vivo, ShcC-deficient mouse significantly decreased $1 \times 1 \text{ cm}^2$ skin defect in wound healing significantly in comparison of the litters.

Conclusions: Both human and mouse bone marrow derived stem or stromal cells express neuronal cell markers. Especially lack of ShcC led to the delayed wound healing. These results may be implicated in nerve-related impaired wound healing.

SURVIVAL AND SIGNALING OF HUMAN MESENCHYMAL STEM CELL IN HIGH DOSE IRRADIATION

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Aim: Bone marrow suppression after high dose irradiation is a well-known fact in the clinical setting. In the bone marrow, the human mesenchymal stem cells (hMSCs) are more resistant to irradiation in comparison to hematopoietic stem cells. Detail cell property of hMSC is not clarified. Therefore, the hMSCs cell kinetics and properties were investigated under high dose irradiation.

Methods: The hMSCs were irradiated at dose of 20 Gy and 60 Gy and human neuroblastoma cell lines (NG108-15) and rat pheochromocytoma cell lines (PC 12) were compared to cell proliferation. The hMSCs were further investigated for signaling.

Results: Both NG108-15 cells and PC12 cells were ceased cell proliferation at dose of 20 Gy, however, the hMSCs mildly proliferated and survived up to 12 weeks. The kinetics of the intracellular signaling of phosphorylated MAP kinase such as phosphor-Erk (p-Erk) is up-regulated and undetectable p38 MAP kinase in the irradiated hMSCs. In the downstream of p-Erk, p90RSK is down-regulated and subsequent phosphorylation of pCREB is also suppressed. The expressions of VEGF, fibronectin and PCNA were all suppressed. On the other hand, phosphorylated Bad (p-Bad) and Akt (Ser 403) were also down-regulated.

Conclusions: With all data on irradiated hMSCs, the hMSC can survive under high dose irradiation (up to 60 Gy) and the intracellular signaling is involved other than Bad-mediated apoptosis.

GREEN FLUORESCENT PROTEIN TRANSFECTION AND BONE DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELL

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Aim: The human mesenchymal stem cells (hMSCs) are useful for regeneration of "mesenchymal" cells and tissues such as bone, cartilage, muscle and fat. The fate after grafting in vivo is unknown thus far. Green fluorescent protein (GFP) is able to track visibly the grafted hMSCs once successfully transfected in the hMSCs.

In order to investigate the cell kinetics and properties, the GFP-transfected hMSCs were further investigated in vivo.

Methods: The hMSCs were transfected with GFP plasmid (pIRES-EGFP) by LIPOFECTAMINE 2000. After confirming the GFP-transfected hMSCs are continued post-cell passage, in vivo grafting in the 4-mm defect nude rat cranial bone defect model.

Results: The GFP-transfection was successful at least up to 3 cell passages. Of the first passaged transfected GFP-hMSCs were grafted in cranial defect of the nude rat. At 7 days after grafting, the superficial layers of the defect independently demonstrated high expression of GFP and mature immunohistological osteocalcin expressions with DAPI (Dihydrochloride) core expressions. The osteogenic transcriptional factor cbfa-1 is identically expressed in the same areas.

Conclusions: The hMSCs are able to be transfected with GFP plasmid by LIPOFECTAMINE 2000. The GFP-hMSCs are able to be tracked in the cranial bone defect model and the bone expression pattern is identical to the independent bone transcription.