



## Abstracts

### The 36<sup>th</sup> Annual Meeting of the Japanese Society for Wound Healing

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Congress President: Takehisa Iwai, M.D., Ph.D. Professor, Department of Vascular Surgery,  
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The following compilation of abstracts represents a partial list of submissions received for presentation at the meeting.

#### GENERAL SESSION 1: TISSUE REGENERATION 1—SCAFFOLD

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##### DEVELOPMENT OF CULTURED SKIN SUBSTITUTE USING A MATRIX OF HYALURONIC ACID SPONGE AND COLLAGEN GEL

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**Purpose:** This study is focused on the development of cultured skin substitute (CSS) using a matrix of hyaluronic acid (HA) sponge and collagen (Col) gel as a matrix. This study is aimed to analyze the biochemical interactions between keratinocytes and fibroblasts.

**Method:** A HA sponge was prepared by freeze-drying an aqueous mixture solution of HA and intermolecular cross-linking agent. The cell suspension of human fibroblasts in culture medium (double concentration of conventional medium) was mixed with an equal amount of collagen solution. The resulting cell suspension (15 ml) was poured into a dish (5 cm in diameter), in which a piece of HA sponge was carefully immersed, and followed by plating it at 37°C for 1 hour to complete gelation. The product was transferred into a dish measuring 10 × 10 cm and then cultured for 1 week to prepare cultured dermal substitute (CDS). According to this manner, CDS was prepared, and followed by seeding keratinocytes on the resulting CDS to prepare cultured skin substitute (CSS). In a similar manner, after gelation of collagen in culture medium without fibroblasts, keratinocytes were seeded on the resulting matrix, and followed by culturing for 1 week to prepare cultured epidermal substitute (CES). The biochemical interactions between keratinocytes and fibroblasts were analyzed by measuring the each amount of VEGF released from CDS, CES, and CSS using ELISA.

**Result:** The results in ELISA showed that the amount of VEGF released from CSS was higher than the total amount of VEGF released from CDS and CES. This finding suggests that both fibroblasts and keratinocytes are stimulated by each other to release more amount of VEGF in vitro. In a histological observation, laminin was found, but type  $\gamma$  collagen was not appeared beneath the stratified keratinocyte in CSS prepared under the given conditions.

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##### DEVELOPMENT OF WOUND DRESSING COMPOSED OF HYALURONIC ACID AND ARGININE

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**Purpose:** This study is designed to develop a spongy type of wound dressing composed of hyaluronic acid and L-argine and then evaluate histologically the efficacy of this wound dressing in animal tests using rat.

**Method:** The spongy type of wound dressing (HA sponge) was prepared by freeze-drying an aqueous solution of high molecular weight hyaluronic acid and intermolecular cross-linking agent (group I). Second version was prepared by freeze-drying an aqueous solution of low molecular weight hyaluronic acid combined with a sheet of HA sponge (group II). Third version was prepared by freeze-drying an aqueous solution of low molecular weight hyaluronic acid and arginine combined with a sheet of HA sponge (group III). Each sponge was

applied on a full-thickness skin defect (35 mm in diameter) in dorsal region of SD rat, over which a polyurethane film dressing and conventional gauze dressing were used to fix each sponge. The wound size was measured and the wound conditions were evaluated histologically 1 week later.

**Results:** A part of spongy matrix remained 1 week later. The wound size decreased in a following order; group I, group II, and group III. The histological evaluation showed that group III promoted highly vascularized granulation tissue formation and well reepithelization from the wound margin.

**Conclusion:** The wound dressing (group III) developed in this study is designed to promote wound healing by the first function with low molecular weight hyaluronic acid and arginine dissolved in an exudate fluid, and followed by the second function with cross-linked high molecular weight hyaluronic acid during a process of biodegradation. In practice, the wound dressings (group III) demonstrated the healthy granulation tissue formation.

#### GENERAL SESSION 2: TISSUE REGENERATION 2

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##### NICOTINE PROMOTES WOUND HEALING

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**Aim:** The adverse effects of smoking on wound healing of the skin are known clinically. Recently, an endogenous cholinergic pathway for angiogenesis mediated by endothelial nicotinic acetylcholine receptors was discovered. The objective of this study was to investigate the appropriate concentration of nicotine at which angiogenesis and wound healing were accelerated.

**Methods:** Experiments on tube formation were conducted using an Angiogenesis Kit. Basic fibroblast growth factor (10 ng/ml) and nicotine (10-10M, 10-8M, 10-6M, 10-4M, 10-2M) were added to the conditioned medium. Full thickness skin defects (8 mm) were created on the dorsum of C57BL mice and a silicone sheet (8 mm) was sutured. PBS (10  $\mu$ l), bFGF (1  $\mu$ g), and nicotine (10-1M, 10-4M, 10-7M) were topically injected for 7 days.

**Results:** Significant differences of area and length of newly formed tube were seen between the control group and the bFGF and 10-10M of nicotine groups. The wound area was significantly decreased in the wound treated with bFGF and with 10-4M of nicotine.

**Conclusions:** Nicotine accelerated angiogenesis and promoted wound healing, though the effect was not more than that of bFGF.

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### INTRAMUSCULAR OSTEOINDUCTION AND CARTILAGE FORMATION BY IMPLANTATION OF rhGDF-5 WITH TYPE I COLLAGEN

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**Objective:** Bone morphogenetic protein-2 (BMP-2) implanted in soft tissue induces ectopic bone formation, making it promising for clinical application in the reconstruction of bone defects and enlargement of bones. Like BMP-2, growth/differentiation factor 5 (GDF-5) belongs to the TGF- $\beta$  family, and reportedly plays an important role in cartilage development and differentiation. In this study, we implanted GDF-5 in rat leg muscle, and evaluated its *in vivo* osteochondrogenesis-inducing activity by histological and X-ray examinations. **Methods:** GDF-5 (0, 100, 300, and 500  $\mu$ g) and the carrier type I collagen were mixed, and the mixture was implanted into rat leg muscle. Two weeks later, the site of implantation in the muscle was examined by soft X-ray, and the muscle tissue including the implantation site was excised, and examined histologically by HE staining, type I collagen immunostaining, Alcian blue staining, and von Kossa staining.

**Results:** The GDF-5 0 and 100  $\mu$ g groups showed no osteochondrogenesis induction. The GDF-5 300  $\mu$ g group showed aggregates of cartilage and some bone tissue in the carrier. The GDF-5 500  $\mu$ g group revealed bone with a small amount of bone marrow and no cartilage.

**Discussion:** These results indicate that GDF-5 induces osteochondrogenesis or osteogenesis, and suggest the possible application of GDF-5 in future regenerative medicine.

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### THE EFFECT OF CANDIDA ALBICANS INFECTION ON DUODENAL ULCER HEALING IN RATS

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**Background and purpose:** It was reported that about 44 percent of the patients with gastroduodenal ulcer perforation had Candida positive in clinically and Candida infection aggravated the duodenal ulcer experimentally. Furthermore, Morishita et al. reported that the curability of gastroduodenal ulcer with Candida infection was low, compared to Candida negative gastroduodenal ulcer. The purpose of this study was to examine whether or not Candida albicans (C. albicans) infection affect duodenal ulcer healing in rats.

**Methods:** Male Wistar rats (7 weeks old) were divided into the Candida group and the control group. All rats were administered with cysteamine after anesthetization on Day 1. C. albicans in 0.5 ml saline was administered to the rats in Candida group from Day 3 to 6, whereas the saline in the control group. The rats were killed on Day 7. The depth of ulcer, the ulcer area and ulcer scar in the duodenum were assessed between the two groups. The immunohistochemistry for vascular endothelial growth factor A (VEGF-A) was performed and the number and area of the VEGF-A positive cell around the ulcer base were compared between the two groups.

**Results:** On Day 7, the incidence of duodenal ulcer in the Candida group and the control group were 70.4%, 33.3%, respectively. There were significant differences on duodenal ulcer area (7.2 Vs 4.7 mm<sup>2</sup>), ulcer scar rate (3.7% Vs 25.0%) between the Candida group and the control group. The number of the VEGF-A positive cell and the area of the VEGF-A positive expression were lower in the Candida group than those of the control group.

**Conclusion:** It was shown that Candida infection delayed the wound healing of duodenal ulcers with low expression of VEGF-A in cysteamine-induced duodenal ulcer in rats.

### GENERAL SESSION 3: WOUND MANAGEMENT

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### TREATING IRRADIATED WOUND WITH BONE MARROW ASPIRATE DERIVED PLATELET RICH PLASMA

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**Aim:** The role of radiation, such as cancer therapy and interventional radiological treatment became indispensable in clinical practice. High dose irradiation may compromise wound healing. The population of cells with high turn over rate, including so-called stem cells, which plays very important role in wound healing, is highly vulnerable to the irradiation. Wipe out of those vivid cells can be considered as the cause of wound healing troubles.

Bone marrow aspirate was processed under the protocol to process platelet rich plasma (PRP) from peripheral blood. We call this concentrate "bm-PRP". We previously reported that "bm-PRP" contains not only concentrated platelets but also concentrated nucleated bone marrow cells. Bone marrow cells, including so-called mesenchymal stem cells, have been reported to be useful in angiogenesis for ischemic heart or limb and in wound bed preparation. Three previously irradiated patients were successfully treated with "bm-PRP".

**Case 1:** A 69 year-old-male underwent Miles' operation after 50Gy irradiation. Anal wound did not heal. "bm-PRP" was injected and a posterior thigh flap was transposed. The flap adapted well to the irradiated floor and the wound was closed.

**Case 2:** A 12 year-old-male, previously 63Gy irradiated and ophthalectomy, underwent malar osteotomy. The incision wound on his lateral canthus did not adapt. "bm-PRP" was injected and temporal fascial flap was transposed. The wound healed.

**Case 3:** A 74 year-old-male underwent 60Gy irradiation to treat laryngeal carcinoma. Anterior wall of trachea was resected with thyroid. "bm-PRP" was injected into the area adjacent to the defect. Ear cartilage was transplanted and skin flap including the cartilage was flipped over the tracheo-skin defect three weeks later. The defect was covered successfully.

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### EFFECT OF THE LOAD CAUSED BY ATTACHING A BANDAGE AT WOUNDS

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Generally, when someone injured, first-aid would be done by attaching a bandage and protecting the part of wound. The time until the skin is regenerated and is completely cured, may be dependent on the size of the part of wound and/or its condition.

The process of curing also depends not only on the medical factor, but also on physical stimulus. The material characteristics of the bandage have an influence on stress to the part of wound when there is a physical stimulus by moving around the parts of wound.

Then, we focused on the backing characteristics of bandages and have measured the stress to the part of wound, a value of blood flow, shear force with either an elastic bandage or non-elastic one in order to examine the ideal adhesive plasters. Regarding the stress to the part of wound, a tensile test instrument was used and three samples are measured. One is with elastic backing and elastic pad, the second one is with non-elastic backing and non-elastic pad and the third one are with elastic backing and non-elastic pad. Next, we have measured the shear force and the normal stress to the part of wound when joint parts are bent while a bandage is attached by using a simple measuring instrument of shear force and normal stress. Moreover, we have measured the value of blood flow of the part of wound with bandage.

By this research, we achieved the knowledge that elastic backing is preferable to reduce the stress to the part of wound. It is necessary to consider the environment for improving the curing ability of the part of wound, for instance, to keep the value of blood value around the part of wound high.

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### TREATMENT FOR PRESSURE ULCERS WITH MIXTURE OF SKIN PIECES AND OINTMENTS WITH A FILM DRESSING

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**Introduction:** We reported the effect of film dressing therapy with mixture of some ointments and excised skin pieces (FMS) for treatment of pressure ulcer.

**Case 1:** An 81-year-old male with cerebral infarction presented to us with a Stage III left trochanteric ulcer of 3 years duration, measured 7.0 by 6.0 cm with undermining of 1.0 cm and a thick epibole of 3 mm in all directions. After film dressing therapy initiated, softened edges were excised. The skin pieces and some ointments were placed in the cavity of the wound, and packed with a food-wrap. Donor sites applied petrolatum with a film dressing, epithelized in about 10 days after surgery. It took two months to heal the cavity, and five months to close the ulcer after several times of FMS.

**Case 2:** An 87-year-old female with a fractured hip developed a Stage IV pressure ulcer measured 7.0 by 5.0 cm on the sacrum. Two months after conventional surgical treatments and film dressing therapy, buried chip skin graft and/or FMS were carried out several times. Donor sites were treated as mentioned above. Five months later, the wound was healed.

**Conclusions:** These results indicate that FMS is effective treatment for skin ulcers worsened and failed to improve.

### GENERAL SESSION 4: KELOID

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### RELATIONSHIP BETWEEN THE DISTRIBUTION OF MYOFIBROBLASTS AND STELLAR SCAR FORMATION DUE TO THE CONTRACTION OF SQUARE WOUND

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Square skin wounds can heal to form a stellar scar with four protrusions at the four angles, whereas circular wounds can heal to form an ellipsoid scar; it is not clear why these differences occur and this study aimed to clarify this phenomenon. Two square or circular full-thickness skin wounds were made on the dorsum of mice, and covered with hydrocolloid dressing. They were observed from day 0 to 15 after wounding, and prepared for paraffin sections stained with anti  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) antibody to detect myofibroblasts (MF). The square wound was transiently enlarged by edema and skin tension on day 3. This phenomenon caused the angles of the wound to become round, thus, the square form became more circular. Thereafter, the wound contracted rapidly and the circular form was maintained to day 11. On day 11 distinct angles appeared again in which MF were fewer than in any other section. Short protrusions from the angles on diagonals were apparently formed on day 15 when MF almost disappeared, and a stellar scar was formed. This indicates that the earlier disappearance of MF from the angles of the square wound causes the square wound to heal to form a stellar scar.

### GENERAL SESSION 5: DRESSING—OPHTHALMOLOGY

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### TREATMENT OF A THIGH SKIN DEFECT USING A TRANSLUCENT COVERING SHEET IN A LONG-TERM STEROID TREATED CASE

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We usually use the skin graft or flap reconstruction technique for relatively wide skin defect of the thigh. It is useful for protecting infection and improving the quality of life.

However in cases of the patients using steroid hormone for 10 years or more, it is difficult to use the conventional plastic surgical technique for the wound, because of skin thinning, retarded wound healing process and poor skin take of the grafted skin. These are the reason why we hesitate to use surgery in such cases.

A 43-year-old women who had been on heavy steroid medication had a relatively wide skin defect of the front thigh due to contusion hematoma. Surgery seemed difficult and the patient also was apprehensive.

So, we use translucent wound covering sheet for healing and obtained a good result after 6 months.

The translucent wound covering sheet we use is VIEWGEL (Taiho pharmaceutical co.) composed of polyethylene support, hydrogel water suction portion and polyethylene liner.

After applying this sheet to the wound, it is easy to see the state of the wound and the reduction in time of the complaint. We think it is useful to apply the Viewgel translucent wound covering sheet instead of the surgery for skin defect treatment in case like this heavy user of steroid.

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### TWO CASES OF EXPOSED HEPATIC ARTERY INFUSION PUMP SALVAGED WITH LOCAL FLAP RECONSTRUCTION WITHOUT CHANGE OF CATHETER

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**Introduction:** For hepatic artery infusion pumps exposed because of hematoma or infection, all systems including the catheter and pump need to be removed and new systems buried at other sites. We salvaged two cases of difficult catheterization without changing the catheter, but by changing only the pump and by local flap reconstruction.

**Case 1:** One month after a hepatic artery infusion pump was buried in the thigh of a 78-year-old woman, necrosis of the skin because of hematoma was successfully managed by changing only the pump and by lomboid flap reconstruction, without changing the catheter.

**Case 2:** Five months after a hepatic artery infusion pump was buried in the thigh of a 64-year-old woman, necrosis of the skin because of hematoma and infection was managed by changing the pump only, not the catheter, and by rotation flap reconstruction.

**Results:** Both patients are doing well. Because of the immediate arterial infusion after the surgery, the planned carcinostatic treatment was not interrupted. By our method, infusion to the target artery was more ensured than by changing the catheter. Adequate debridement around the pump together with part of the catheter and reconstruction with a flap of sufficient blood flow were essential.

**Conclusion:** In cases of difficulty of catheterizing target arteries, reconstruction with a local flap together with adequate debridement is one of the choices of treatment.

## SYMPOSIUM 1: ACCELERATING WOUND HEALING— PRESENT AND FUTURE

### Y1-1

#### **bFGF PROMOTES APOPTOSIS AND INHIBITS EXPRESSION OF ALPHA-SMOOTH MUSCLE ACTIN, LEADING TO REDUCTION OF WOUND CONTRACTION IN ARTIFICIAL DERMIS**

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To clarify the mechanisms underlying declines in wound contraction caused by basic fibroblast growth factor (bFGF) and the role of autologous fibroblasts in modulating wound healing, we have examined expression of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) and apoptosis using collagen sponges with and without bFGF (1 mg) and/or fibroblasts ( $1 \times 10^6$  cells/cm<sup>2</sup>) applied to experimentally produced full-thickness skin wounds in rats ( $n = 10$  for each group). At 7 days postoperatively, wounds filled with fibroblast-seeded collagen sponge (fibroblast-seeded group) displayed a greater area of collagen sponge and a smaller area of fibroblasts compared to control wounds filled with collagen sponge alone (control group). By day 14, wounds filled with bFGF-treated collagen sponge without fibroblast seeding (bFGF group) displayed decreased  $\alpha$ -SMA expression and significantly increased apoptosis compared to other wounds. Double staining revealed that apoptosis in  $\alpha$ -SMA-positive fibroblastic cells was significantly increased in the bFGF group, suggesting that bFGF treatment is a potent stimulator of myofibroblast apoptosis. Morphometric analysis demonstrated the significant decrease in the level of wound contraction and the degree of mature collagen bundle formation in the bFGF group by day 42. These results suggest that bFGF administration to artificial dermis promotes apoptosis of  $\alpha$ -SMA-positive fibroblastic cells and inhibits  $\alpha$ -SMA expression in the treated wound, thus reducing wound contraction.

### Y1-3

#### **DEVELOPMENT OF REFRACTORY ULCER TREATMENT WITH ADIPOSE TISSUE-DERIVED CELLS**

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**Purpose:** As the latest treatment for refractory ulcers related to chronic arterial occlusion or diabetes, a procedure using bone marrow cells is applied in clinical practice. We have investigated the cultured dermis using adipose tissue-derived stem cells, which function as stem cells, as demonstrated for bone marrow cells. In this study, we isolated cells including stem cells from adipose tissue, and examined the possibility of refractory ulcer treatment with collagen sponge and adipose tissue-derived cells.

**Methods:** Cells including stem cells were isolated from the abdominal adipose tissue of mice with diabetes, and disseminated on collagen sponge. A dermal defect wound was prepared on the back of the same animals, and the collagen sponge containing adipose tissue-derived cells was implanted. As a control substance, collagen sponge alone was also implanted. After 1 and 2 weeks, we examined whether wound healing was achieved.

**Results:** The collagen sponge containing adipose tissue-derived cells macroscopically and histologically achieved better wound healing compared to collagen sponge alone.

**Discussion:** Implantation of the collagen sponge containing adipose tissue-derived cells promoted wound healing in the mice with diabetes. Our results suggest that implantation of collagen sponge containing adipose tissue-derived cells collected from diabetics is useful for treating refractory ulcers.

### Y1-4

#### **NEURAL REGENERATION AND ACCELERATION OF WOUND HEALING BY MESENCHYMAL STEM CELLS**

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**Aim:** The neuronal involvement for local wound healing is clinically well documented in impaired wound healing, which is observed in diabetic and vascular diseases. Mesenchymal stem cells of the human (hMSCs) are able to differentiate into the nerve. The effect for nerve regeneration and wound healing is tested in an in vivo nerve-severed and hMSC grafted lower limb model.

**Methods:** The fluorescent labeled hMSCs were grafted in a saphenous nerve-severed and femoral vessel ligated nude rat model for up to four weeks.

**Results:** The nerve recovery was significantly greater in hMSC-grafted group by a nerve conductance method. The tissue blood flow was increased in a time-dependent manner. The wound healing of the planter paw was increased in the hMSC-grafted group. More neural-differentiated cells were observed in immunohistochemistry with co-localization of fluorescent labeled cells.

**Conclusions:** The hMSCs are able to contribute to nerve-severed and blood blocked lower limb model. The wound healing is significantly accelerated with association of the nerve regeneration.

### Y1-5

#### **EFFECTIVENESS OF CLINICAL TREATMENT WITH AUTOLOGOUS PLATELET-RICH PLASMA IN PLASTIC SURGERY**

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**Aim:** The purpose of this study was to evaluate a new technique of harvesting and preparing autologous platelet-rich plasma (PRP) and to evaluate its effectiveness in plastic surgery.

**Methods:** PRP was prepared in about 20 minutes anywhere in clinical fields from autologous blood using a compact, portable, automated platelet concentrate closed system (MAGELLAN, Medtronic.). Three patients underwent this treatment. Case 1: 63-year-old female had been treated for rheumatoid arthritis with steroid and chronic nonhealing leg ulcer past for 6 months. She underwent two times of PRP (gel sheet) treatment every one week after controlling wound infection. Case 2: 25-year-old female with chronic skin ulcer and exposure of periost in the right foot caused by traffic accident was treated with PRP which injected under full-thick skin graft preserved subcutaneous vascular network 2 weeks after debridement and covering with artificial dermis. Case 3: 22-year-old female with fibrous dysplasia in the left maxilla was sprayed with PRP on the wound surface for hemostasis and promoting wound heal after wide resection of bone tumor.

**Results:** The platelet concentration of PRP increased varied from 3 to 9 times above baseline. All of three satisfactorily recovered with no adverse effects.

**Conclusions:** This study has revealed that the use of PRP as a tissue repair and regeneration agent, and hemostatic agent after appropriate wound preparation has resulted in improved outcomes in plastic surgery.

## Y1-6

**EXAMINATION OF PRP THERAPY (SKIN GRAFT ON PRP GEL SHEET THERAPY) AT ISCHEMIC(ASO)CALCANEAL ULCER**

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We announced that we treated lt calcaneal ischemic (ASO) ulcer with PRP Therapy (combination PRP gel and skin graft) and gained wound closure.

Patient was 76 yeas old male. At first blister became at lt calcaneous state. But patient didn't care it. Next It became necrosis and Dermatologist debrided it but ulcer didn't getting better. Pt entered in our Plastic & Reconstructive Surgery hospital on March 2006. ABPI score was 0.42 at Lt Lower Leg, 0.54 at Rt Lower Leg. CT-Angiography showed about 30% occlusion at superficial femoral artery, about 50% occlusion at popliteal artery. anterior&posterior tibial artery/peroneal artery was thin but lead to distal. Surgical & chemical debridement was performed and used silver sulfadiazine cream (Silvadane/Geben Cream) because Pus culture was Pseudomonas Aeruginosa (sensitive;CAZ/PIPC).

Wound bed preparation peformed, we collected arterial blood 70cc, peformed 8/1000 inch 3 times Mesh skin graft. We gained 5.73cc Platelet Rich Plasma, 24.6cc Platelet Poor Plasma. We putted PRP, mesh skin graft next PPP on wound and fixted tie over.

Condense rate was 3.12 times on platelet, 3.64 times on WBC, 3.38 times on neutrophil.

We removed tie over at post operative 6 day, 90% skin graft was taken and didn't show infection sign at wound. Conclusively PRP/PPP lead No infection, Stability at skin graft, early Epithelization on mesh skin graft. PPP was used protection on bacterial infection and inflammation. We made good use of patient's blood by PPP.

## Y1-7

**TREATMENT OF SKIN DEFECTS BY ONE-STEP GRAFTING PROCEDURE OF ARTIFICIAL DERMIS AND SPLIT-THICKNESS SKIN**

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**Aim:** The purpose of this study was to achieve immediate skin grafting following the artificial dermis (AD) grafting. To achieve the task, allogenic cultured endothelial cells, fibroblasts and autogeneous PDWHF (platelet derived wound healing factors) were employed when the AD were grafted. On a basis of experimental study, we performed clinical trials in 2 cases of extensive burns and a case of post burn deformity.

**Methods:** Dermal microvascular endothelial cells and fibroblasts were isolated from human skin sample and cultivated for cryopreservation. The PDWHF was prepared from the autogeneous platelet. Prepared PDWHF solution was infiltrated into the AD (TERUDERMIS<sup>®</sup>, 3 mm thickness, collagen sponge alone without silicone sheet, TERUMO Co., Japan). Microvascular endothelial cells and dermal fibroblasts recovered from cryopreservation were also prepared. This cell suspension was dispersed on the AD filtrated with the PDWHF. The prepared AD involving the PDWHF and the cultures were applied on the skin defects. Immediately after the AD grafting, 10/1,000 inch thickness autogeneous skin (1:3 mesh in 2 cases of burns, sheet in a case of post burn deformity) were grafted on the AD. Ten days after surgery, the dressing was opened and a take of the skin was evaluated.

**Results:** The grafted skin was well taken in all cases. Histological examination revealed that vobine collagen tissue lasted in the grafted artificial dermis for at least 1month after the surgery, however it disappeared in 2 years after the surgery.

**Conclusions:** This method may be an alternative method of skin reconstruction using the AD in the cases of extensive skin defects such as severe extensive burns.

## Y1-8

**EFFECTIVENESS OF SHORT-CONTACT TOPICAL TRETINOIN IN PROMOTING WOUND HEALING FOR FULL-THICKNESS WOUNDS**

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**Aim:** The topical tretinoin (all-trans-retinoic acid; a derivative of vitamin A.) therapy for patients with photo-aging has spread among the aesthetic and dermatological clinics in Japan. Various biological effects of the tretinoin are well documented.

Recently, Paquette has reported that short contact daily application of tretinoin topically improved the healing in five chronic leg ulcers.

We have investigated the effects of short-contact topical application of tretinoin for full-thickness wounds on db/db mice.

**Method:** We made two full-thickness wounds to the skin on the backs of our 16 male db/db mice. On each mouse, 0.1% tretinoin aqueous gel was applied to one wound for five minutes daily for five successive days while non-tretinoin aqueous gel was applied on the other wound (control). Then we assessed the results of healing by observing the Measurements of the size of the wound, the thickness of granulation and the density of capillary vessels in granulation by histological findings.

**Results:** The mean (SD) percentage surface area unhealed before and after treatment were 0.88 (0.3) and 0.64 (0.15). In tretinoin-treated mice and non-treated mice (controls), the mean (SD) thicknesses of granulation were 1.383 (697) mm and 683 (413) mm, the density of capillary vessels in granulation was 12.2 (5.5)% and 5.7 (3.9)%, respectively.

Differences between the two groups were significant for each variable.

**Conclusions:** Wound healing was accelerated with short-contact topical application of tretinoin in db/db mice.

**References:** Paquette D, Badiavas E, Falanga V. Short-contact topical tretinoin therapy to stimulate granulation tissue in chronic wounds. J Am Acad Dermatol 2001;45:382-6.

**SYMPOSIUM 2: SCARLESS WOUND HEALING**

## Y2-4

**BASIC FIBROBLAST GROWTH FACTOR INDUCED MYOSIN II REGULATORY LIGHT CHAIN PHOSPHORYLATION ON FIBROBLAST BUT NOT ON MYOFIBROBLAST**

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Recent clinical reports have pointed out that basic fibroblast growth factor (bFGF) promotes scarless wound healing. The present studies were carried out to elucidate the mechanism. Previous studies reported that 3–10 days of transforming growth factor  $\beta$  (TGF $\beta$ ) treatment were required to activate fibroblasts to express the myofibroblast phenotype *in vitro*. Then we employed this technique to obtain myofibroblast. bFGF well stimulated fibroblast-collagen gel contraction but not myofibroblast-collagen gel contraction. Subsequent studies were carried out to determine if co-stimulation of fibroblasts with TGF $\beta$  and bFGF affected their ability to express the myofibroblast phenotype. In these experiments, fibroblasts in monolayer were incubated with or without 1 ng/ml of bFGF in the presence of 10 ng/ml of TGF $\beta$ , harvested, and then tested for the expression level of  $\alpha$ smooth muscle actin ( $\alpha$ SMA). Levels of cellular  $\alpha$ SMA increased after 2–4 days of TGF $\beta$  treatment alone and were consistently elevated after 5 days with unchanged levels of total actin. However it is of note that levels of cellular  $\alpha$ SMA increased after 4–6 days of bFGF and TGF $\beta$  treatment. Other experiments were carried out to test the effects of bFGF on myosin II regulatory light chain (MLC) phosphorylation on fibroblasts and myofibroblasts. After 30 min of bFGF, levels of diphosphorylated MLC were highest in fibroblasts. In contrast, levels of diphosphorylated MLC were still elevated in myofibroblasts spontaneously, and were consistently unchanged even if stimulated with bFGF. Our results indicated that bFGF had certain roles on the expression of myofibroblast phenotype by fibroblasts and MLC phosphorylation by myofibroblasts.

### SYMPOSIUM 3: ARTERIOSCLEROSIS OBLITERANS— PRESERVATION VS. RECONSTRUCTION

#### Y3-1

#### HYBRID TREATMENT FOR CRITICAL LIMB ISCHEMIA USING SKIN PERFUSION PRESSURE (SPP) AS WOUND CARE MANAGEMENT ~TEN CASES REPORT

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**Background:** Recently, numbers of patients with peripheral arterial disease (PAD) have been increasing in Japan. So, a proper wound care management for critical limb ischemia (CLI) is important.

**Aim:** The purpose of this study is to evaluate whether these critical limbs can be salvaged by hybrid treatment with SPP management. We review our cases.

**Methods:** We experienced ten patients with CLI due to PAD. We performed both bypass operation and percutaneous transluminal angioplasty (PTA) after SPP measurement. After improving SPP data, we performed local operations (surgical debridement and skin graft or minor amputation) and all wounds were healed.

**Results:** When the patients with CLI could not be given enough blood supply to heal wounds only by bypass operation or only by PTA, they had hybrid treatment by both, local operations (debridement or minor amputation) timely at the appropriate level.

**Conclusions:** Vascular surgeons do bypass operations and interventionalists perform PTAs individually. But hybrid combination treatment with SPP management is a reliable procedure that has great possibility for CLI to cure.

#### Y3-3

#### TREATMENT STRATEGY FOR LIMB-THREATENED ISCHEMIC ULCER OR GANGRENE

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Among several procedures to treat critical limb ischemia (CLI), bypass surgery has the most potent effects to cure the ischemic ulcer or gangrene. We reviewed clinical results of our aggressive complete revascularization.

**Patients and Methods:** We have experienced 161 critical ischemic limbs with tissue loss in 153 patients since 1996. Sixty-nine percent of patients have diabetes and 36% was hemodialysis (HD)-dependent renal failure.

**Results:** Four limbs (2.5%) underwent primary major amputation due to extensive infection or tissue defect beyond ankle joint. Only one patient was performed gene therapy using hepatocyte growth factor plasmid because of no graftable artery. The rest of limbs (97%) were able to undergo bypass surgery with or without catheter intervention. Since most of diabetic patients have graftable paramalleolar arteries, 98 limbs underwent paramalleolar or pedal bypasses. Eleven limbs which had large size of defect underwent free flap transfer such as rectus flaps and latissimus dorsi flaps following bypass surgery. Cumulative limb salvage rate was 94% in 5 year. However, it takes long term to cure diabetic tissue loss especially in HD patients even in using topical growth factor and vacuum assisted closure.

**Conclusions:** More than 90% of CLI can be salvaged by direct revascularization. Further development of topical treatment facilitating wound healing after bypass surgery is needed to cure ischemic tissue loss in a minimum time and minimum cost.

#### Y3-4

#### PRESENT STATUS OF THE TREATMENTS FOR ISCHEMIC ULCER AND GANGRENE IN OUR DEPARTMENT

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**Materials and Methods:** Ninety six limbs with ischemic ulcer or gangrene (81 patients), who were admitted since January 2005, were evaluated. There were 60 male and 21 female patients. The age ranged from 25 to 92 years old with an average of 68 years old. Twenty nine patients were complicated with diabetes and 22 were on hemodialysis. Ischemic lesions were located at upper extremities in 9, lower limbs in 88 (including 4 legs, 24 feet and 60 digits).

**Results:** Two limbs with extended gangrene or infection into the leg were primarily amputated. Thirteen ischemia ulcers limited in digits treated conservatively and one performed with sympathectomy were all cured. Among 47 limbs treated with arterial reconstructions (9 endovascular surgery, 38 open bypass procedures), 41 were cured and 2 were not cured with one major amputation. The patients with the remaining 4 limbs were dead at discharge. Thirty three limbs did not have an indication for arterial reconstruction and were treated conservatively. Among those, 16 were cured and 10 were not cured with 4 major amputations. There were 5 hospital deaths.

**Conclusion:** Ischemia limited in digits had a good prognosis. The patients with more extended ischemia had worse prognosis if they were not the candidates for arterial reconstructions. To improve the limb salvage rate, the arterial reconstruction should be done, however, there are a considerable number of the patients with severe diffuse arterial lesions or systemic complications, which preclude surgery, and it seems to be necessary to sophisticate the conservati

### SYMPOSIUM 4: PATHOGENESIS AND TREATMENT OF CRURAL ULCER

#### Y4-1

#### INTRACTABLE SKIN ULCERS WITH CHRONIC RHEUMATOID ARTHRITIS

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**Background:** It is generally said that the number of the patients with chronic rheumatoid arthritis (RA) is about seven hundred thousand. Skin ulcers are one of the many extraarticular manifestations and tend to be intractable. However, it is unknown why RA causes skin ulcer and the reason behind their intractable.

**Aim:** To investigate the etiology and treatment methods of intractable skin ulcers with RA.

**Methods:** We reviewed 11 RA patients with intractable skin ulcers at Kobe university hospital and Shin-suma general hospital during April 2004–August 2006 (n = 11, sex; male = 1 female = 10, region; lower limb, average age; 63.5).

**Results:** It was shown that diabetes (n = 2), peripheral arterial disease (PAD) (n = 3), chronic hepatitis C (n = 2) and cryoglobulinemia (n = 1) contributed to the poor healing of skin ulcers. We found that toe deformation causes skin ulcers and steroid treatment for RA made them intractable (n = 8). The clinical outcomes were; 4 patients had healed ulcers, 5 patients needed continued treatment, 2 patients required amputation of their lower limbs (1 patient died).

**Conclusions:** The keypoints of treating intractable skin ulcer include the treatment of RA and its complications, improvement of peripheral circulation, proper tapering the dose of its immunosuppressants, the closing ulcer after well wound bed preparation, and the appropriate foot care and footwear preventing getting worth and recurrence.

Y4-4

**BLUE TOE SYNDROME WITH FOOT ULCERS**

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**Background:** Blue toe syndrome is characterized by a suddenly onset of painful livedo reticularis with purple discoloration of foot, secondary to microathero-embolism from fragile atherosclerotic plaques. Although blue toe syndrome can occur spontaneously, it has been increasingly recognized as an iatrogenic complication from the involvement of any vascular procedure.

**Methods:** We reviewed eight patients (seven men and a woman; 64–88 years) of blue toe syndrome with foot ulcers.

**Results:** It occurred spontaneously in four patients and iatrogenic in other four. Two foot ulcers healed with conservative therapies, three foot ulcers underwent minor amputations and three patients died before their wound healings. We performed LDL-apheresis in three patients and applied corticosteroids in two patients. During this period, renal insufficiency developed in five patients and ischemic enteritis in one patient. Four patients died.

**Conclusions:** Blue toe syndrome is a multisystemic disorder. Micro-atheroembolic occlusions result in multiorgan system failure and death. It is necessary to care in performing interventional procedure to patients who have risk factors of blue toe syndrome.

**SYMPOSIUM 5: MANAGEMENT OF SURGICAL WOUNDS**

Y5-1

**ANALYSIS OF SPECIAL FEATURES OF VARIOUS FILM DRESSINGS**

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**Introduction:** Film dressings have various special features such as water vapor transmission rate (WVTR), adhesiveness of material, and sterility.

**Methods:** Three kinds of film with various WVTR (700, 2200, 11000 g/m<sup>2</sup>/day) were placed on suture wounds of abdominal mid-line surgical incisions immediately after the operation, and the volume of exudate was observed for one week.

**Results:** With the 700 g/m<sup>2</sup>/day WVTR film, the exudate stayed for one week, but in the 2200 g/m<sup>2</sup>/day WVTR film, the exudate first stayed and then perspired a few days after the operation. With the 11000 g/m<sup>2</sup>/day WVTR film, the exudate perspired on the day after the operation.

In facial injuries, the 11000 g/m<sup>2</sup>/day WVTR film was used for the fixation of alginate dressing. Dressings of high quality were able without leakage of exudate. Film with gel adhesive adhered immediately and more closely to the dry skin of the aged than generally used film with acryl adhesive, and was safely detached from fragile skin, but tended to detach on parts other than skin. The use of sterilized film was found to be not useful on skin with various bacteria.

**Conclusion:** To understand various special features of film dressings and to apply them properly to wounds and at appropriate sites is essential.

Y5-4

**MAKING OF A PSEUDOMONAS AERUGINOSA BIOFILM MODEL IN A RAT SKIN ULCER**

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**Introduction:** In late years biofilm to form as one of the factors in the living body surface and an organization attracts attention obstinateness of bacterial infection symptom. Obstinateness is expected in wound healing by the formation of biofilm. Although there is many it, as for the report of an artificial biofilm formation model in *in vivo*, studies of biofilm in a real animal skin ulcer model are few.

**Aim:** We examined ischemic ulcer (ischemia group) and the biofilm formation in non-ischemic ulcer (control group) this time to clarify a skin ulcer of a blood-stream imperfection state and relations of the biofilm formation by making an ulcer on the skin of dialect which I made to the rat back.

**Methods:** We made a skin ulcer in ischemia department and a non-ischemia department of the male S-D rat back and we gathered an organization in an ulcer 40 hours later 24 hours later dissemination, eight hours later and dyed *P. aeruginosa* PAO1(FITC labeling) in Concanavalin A 24 hours later and observed it.

**Results:** The formation of biofilm was already accepted in the back for eight hours. There was comparatively much formation of biofilm in non-ischemia-related ulcer (control group), and, in comparison with an ischemia-related ulcer.

**Conclusions:** It followed that it was recognized, and the possibility that neutrophile permeation to a wound participated in the biofilm formation in one way or another was suggested. I developed this model more and became a clue to elucidate ischemia and mechanism of the biofilm formation in future.

**References**

1. L.L. Greiner et.al. Biofilm Formation by Neisseria gonorrhoeae: American Society for Microbiology. Vol. 73. 1964–1970.2005.
2. Patricia M. Mertz et al. Model for Studing Bacterial Adherence to Skin Wounds: American Society for Microbiology. Vol. 25. 1601–1604.1987.
3. Kendra P. Rumbaugh et al. Contribution of Quorum Sensing to Virulence of Pseudomonas aeruginosa in Burn Wound Infections. American Society for Microbiology. Vol. 67. 5854–5862.1999.

**SYMPOSIUM 6: PATHOPHYSIOLOGY OF DECUBITUS AND THE LATEST TREATMENT**

Y6-1

**INDICATION OF SURGICAL OPENING OF PRESSURE ULCERS WITH POCKETS**

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While treating patients with pressure ulcers, we often observe undermined area from wound edges, that we call pockets. Pockets might cause wound infection and hinder the healing of wounds. Residual pockets are mainly due to the persistent pressure and shear forces around pressure ulcers. Of cause it is necessary to relieve the pressure and shear forces. Surgical opening of pockets is also sometimes necessary for healing of pressure ulcers.

To clarify an indication of surgical opening of pockets, we compared pressure ulcers with pockets, (1) the spontaneously closed group and (2) surgically opened group, by the size of pressure ulcers, existence of infections and depth of pockets. Between October 2003 and October 2005, 18 pockets of pressure ulcers were closed spontaneously, and 10 pockets were surgically incised. 7 out of the 18 spontaneously closed cases were infected. Whereas 6 out of the 10 surgically opened cases were infected. There was no significant difference.

The average size of pressure ulcers of spontaneously closed cases was 8.2 ± 6.9 (7–300 mm). Whereas the average size of pressure ulcer of surgically opened cases was 112.5 ± 71.7 (40–300 mm). There was statistically significant difference by Mann–Whitney U test (p = 0.03).

The average depth of pockets of spontaneously closed ulcers was 13.2 ± 6.8 (3–25 mm). Whereas the average depth of surgically opened ulcers was 60.0 ± 30.4 (35–120 mm). There was statistically significant difference (p < 0.001). During periods of data collection, there was no pockets with more than 30 mm depth closed spontaneously.

**Conclusions** Our criteria to select the treatment for pockets is following. If the depth of pockets is under 3 cm, we choose conservative treatment. If the depth is over 4 cm, we decide surgical opening of the pockets. If the depth is between 3 and 4 cm, treat conservatively for a couple of weeks, then if there is no improvement, we do surgical opening.

## Y6-2

**DECUBITUS OF PATIENTS WITH SPINA BIFIDA ~ THE PATHOPHYSIOLOGY AND OUR STRATEGY OF THE TREATMENT ~**Fujii M.<sup>1</sup>, Terashi H.<sup>1</sup>, Tahara S.<sup>1</sup>, Tsuji Y.<sup>2</sup> and Kitano I.<sup>2</sup><sup>1</sup>Departments of Plastic Surgery, Kobe University, Kobe, Japan<sup>2</sup>Wound Therapy Center, Shin-suma General Hospital, Kobe, Japan

**Background:** Because of various foot deformities or paralysis, 85% of the patients with spina bifida have decubitus in their lives. But there have been little reports about them.

**Aim:** We review our cases and examine the pathophysiology, and introduce our strategy of the treatment.

**Methods:** We treated eight patients with decubitus due to spina bifida; three males and four females, and the average age was 33. Four ambulatory patients had decubitus with their feet, three patients with wheelchair had with their ischial, and one with their coccygeal areas.

**Results:** Some cases got worse because of less compliance about their decubitus. In addition to the insensate by the paralysis, the most patients did not have enough informations about their decubitus.

**Conclusions:** We should not only treat local therapy for the decubitus but also give any information to the patients in cooperation with other medical facilities or other specialists (for example for shoes). Recently, we educate the patients by showing the patients their actual pressure condition, using Force Sensitive Applications (FSA) system, and enlighten about our knowledge to Spina Bifida Association of Japan by lectures.

## Y6-3

**BURIED CHIP SKIN GRAFT FOR BEDSORES**Koji Nishihori, Ayuko Kono, Mai Ishikawa, Kazuhisa Yokoo  
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**Introduction:** Treatment of bedsores may be complicated by the condition of the patient. Buried chip skin grafts, which can be easily performed in a short period of time, are the treatment of choice for patients with dementia and other psychiatric diseases. Although maintenance of postoperative rest may be difficult, this does not affect graft survival. Herein, we investigated the graft survival rate and wound closure of buried chip skin grafts for intractable bedsores.

**Subjects:** Elderly patients with dementia and other psychiatric diseases with bedsores in the sacral, trochanteric, calcaneal, and other regions.

**Methods:** Since long rests were difficult for patients, surgery time was set at 15 to 20 min. Thin split thickness skin was harvested from the lateral thigh under local anesthesia or using an anesthetic patch or an occlusive dressing technique. After making a U-shaped incision (approximately 7–8 mm) on the granulation tissue and lifting the area as a granulation flap, the thin split thickness skin graft was cut into 5–8-mm chips and buried in the skin flap. The wound was covered immediately after surgery using gauze with antibiotic ointment. Patients required no rest thereafter, and no particular restrictions were placed on postoperative rest.

**Results:** Although survival of the buried chip skin graft could not be initially determined, wound contraction was observed on a daily basis.

**Discussion:** These patients, who were contraindicated for surgery, achieved favorable wound closure following outpatient surgery for bedsores under local anesthesia. Considering the anesthetic risk of surgery and its effects on postoperative lifestyle, buried chip skin grafts are a realistic and useful method with minimal burden and cost for patients with intractable bedsores.

**SYMPOSIUM 7: WOUND HEALING AND EXTRACELLULAR MATRIX METABOLISM**

## Y7-2

**WOUND HEALING IS DELAYED IN MMP-9 AND MMP-13 DEFICIENT MICE**Noriko Hattori<sup>1,2</sup>, Kishi Kazuo<sup>1</sup>, Tastuo Nakajima<sup>1</sup>, Hironari Takaishi<sup>3</sup>, Jeanine D'Armiento<sup>4</sup> and Yasunori Okada<sup>2</sup><sup>1</sup>Departments of Plastic and Reconstructive Surgery,<sup>2</sup>Pathology and<sup>3</sup>Orthopaedic Surgery, School of medicine, Keio University, Tokyo, Japan<sup>4</sup>Department of Molecular Medicine, Columbia University, College of Physicians and Surgeons, New York, USA

**Methods:** We attempted to examine the roles of MMP-9 and MMP-13 in wound healing by comparing the full-thickness cutaneous wounds made on the back of MMP-9 knockout (KO), MMP-13 KO, MMP-9/13 double KO and wild-type (WT) mice using an 8-mm biopsy punch instrument.

**Results:** Macroscopically, wound closure was significantly delayed in MMP-13 KO, MMP-9 KO and MMP-9/13 double KO mice in this order. The rate of re-epithelialization, which was histologically determined by measuring the distance between the leading edges of keratinocyte migration, was significantly delayed at a similar level in MMP-9 KO and MMP-13 KO mice and remarkably in MMP-9/13 double KO mice compared with WT mice. Vascular density in the wound granulation, which was determined by counting CD31-positive endothelial cells, was significantly decreased on day 5 in MMP-13 KO and MMP-9/13 double KO mice compared to WT mice. Planimetric image analysis showed that the areas with myofibroblasts were significantly smaller on day 7 in MMP-13 KO and MMP-9/13 double KO than in WT and MMP-9 KO mice. In addition, granulation tissue appeared to be wider and thinner in MMP-13 KO and MMP-9/13 double KO mice than in WT and MMP-9 KO mice.

**Conclusions:** These data suggest that both MMP-9 and MMP-13 play an important role in keratinocyte migration, angiogenesis and granulation tissue formation in wound healing.

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Y7-4

**REMODELING OF BASEMENT MEMBRANE AND ACQUISITION OF BARRIER FUNCTION IN POSTGRAFTING STATUS OF EX-VIVO PRODUCED ORAL MUCOSAL COMPOSITE EQUIVALENT (EVPOME)**

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**Background:** EVPOME is generated in a serum-free culture system, without the use of an irradiated xenogeneic feeder layer, by seeding human oral keratinocytes onto a human cadaveric dermal equivalent, AlloDerm. We have grafted EVPOMEs in more than one hundred cases with various oral defects.

**Aim:** The aim of this study is to know the wound healing process of EVPOME grafting in detail.

**Methods:** We studied remodeling of basement membrane and acquisition of barrier function in postgrafting status of EVPOME by immunohistochemical stainings with Laminin 1, 5, and Type VII collagen, and with human  $\beta$  defensin.

**Results:** The usual course after grafting with HE staining shows that the rete ridge disappear at 10 days and gradually come to back to normal structure by 4 weeks. Immunohistochemical stainings of basement membrane show that Laminin 1 is positive weakly and Type VII collagen is negative at 10 days after grafting, and then come to back to positive by 4 weeks. Immunohistochemical staining of human  $\beta$  defensin also shows the normal structure by 4 weeks.

**Conclusions:** EVPOME is one of the reconstructive options for oral epithelial defect.

Y7-5

**EFFECT OF PHOTODYNAMIC THERAPY ON COLLAGEN METABOLISM IN KELOID FIBROBLASTS**

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**Aim:** Topical photodynamic therapy using 5-aminolevulinic acid (ALA-PDT) is a therapeutic approach currently under investigation in clinical studies for the treatment of superficial skin tumor. The aim of this study was to investigate the effect of ALA-PDT on collagen metabolism in keloid fibroblasts.

**Methods:** Normal and keloid fibroblasts were treated with 5-aminolevulinic acid and red light. Cell supernatants were taken after PDT to determine protein level of matrix metalloproteinase 1 and 2 and of their inhibitors, tissue inhibitor of metalloproteinase 1 and 2 by enzyme linked immunosorbent assay. Cellular mRNA expression of these proteins and collagen type I and III was measured by quantitative real-time polymerase chain reaction.

**Results:** A significant induction of matrix metalloproteinase 1 protein level was seen after ALA-PDT. The mRNA levels of matrix metalloproteinase 1 was increased after 12 h after irradiation, whereas collagen type I mRNA was strongly decreased following irradiation.

**Conclusion:** These data show that ALA-PDT induce metalloproteinase 1 expression in normal and keloid fibroblasts, while reducing collagen type I mRNA expression. Induction of collagen-degrading enzymes with reduction of collagen production might be responsible for the anti-fibrotic effects of ALA-PDT observed in vivo.