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Congress President: Yoshiaki Tai, Professor and Chairman, Department of Plastic & Reconstructive Surgery and Maxillofacial Surgery, Kurume University School of Medicine

Symposium I Scarless Wound Healing

Moderated by Toshiharu Ishii, Yuu Maruyama

S-I-01

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

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We examined the relationship between the degree of healing and the level of apoptosis in full-thickness- incisional skin wounds, which were treated by conventional suturing with or without intradermal injection of basic fibroblast growth factor (bFGF) (0.1 μ g and 1 μ g/cm of wound). Histological parameters such as the width of wound tissue showed that the degree of granulation formation in the 1 μ g-bFGF-treated group significantly increased on day 7, whereas the degree of scar formation significantly decreased on days 14 and 28. Apoptotic cells significantly increased in the number on day 4 in the 1 μ g-bFGF-treated group compared with that of the control group ($p=0.024$), and decreased on days 14 and 28. These findings suggest that the accelerated apoptosis in the bFGF-treated wounds contributes to the decreased cellularity in inflammatory change through elimination of cells with apoptosis, which resulted also in the reduction of scar formation. In parallel with apoptotic induction, TGF- β 1 and activated caspase-3 positive cells significantly increased on day 4 ($p=0.015$) and day 7 ($p=0.025$), respectively, suggesting the possible role of TGF- β 1 in apoptosis of inflammatory cells. These findings indicate that the early enhanced apoptosis in the bFGF-treated wounds contributes to the decreased cellularity in the granulation tissue.

S-I-03

DIFFERENTIATION OF MESENCHYMAL PROGENITOR CELLS DERIVED FROM RAT BONE MARROW

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We showed mesenchymal progenitor cells(MPC) contain various cells, and the differentiatinal capacities and effects on wound healing were different each other. Bone marrow of the femur of adult F344 rat was suspended into culture medium and plated on a plastic dish.10-15 passaged cells were cloned in 100 types cells. For investigation of the differentiatinal capacity, the cells were treated by differentiatinal medium. The capacity of myogenesis was examined immunohistochemically with anti-skeletal muscle myosin antibody. Osteochondrogenesis were investigated by alcian blue staining and adipogenesis by oil red O staining. The 53% of cells showed myogenesis, 78% osteochondrogenesis and 100% adipogenesis. The one type of cell showed amazing adipogenesis pattern, which we named "O" cell. Next we injected MPC and this "O" cell into the rats' dorsal skin, and 1 cm full thickness incisional wounds were made immediately after. 2 weeks later, wounds were harvested and examined histologically. The wounds transplanted with high dose of MPC healed with very fine scar and collagen fibers were thick and aligned like normal dermis. But by transplanting the "O" cells, wounds healed with ordinary scar formation. By tansplantating mesenchymal progenitor cells, lesion healed with less scars. And there was a possibility that the MPC responded to the wound healing and regenerated dermal structure nearly normally. But the wounds healed not well by transplanting just "O" cell although it possesses the amazing differentiatinal capacity. The data demonstrate that plural types cells were necessary for wound healing.

S-I-05

INCREASED LEVELS OF CELL DIVISION CYCLE 25A PROTEIN IN KELOID LESIONS

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Aim: Keloid lesions develop as a result of abnormal growth of dermal fibroblasts after the injury. On the other hand, cell division cycle (Cdc) 25 is a family of phosphatases that activate the cell cycle regulating cyclin-dependent kinases. The three members of this family, Cdc25A, Cdc25B, and Cdc25C act in different phases of the cell cycle. In this study, we examined the degree of expression of these phosphatases in keloid fibroblasts.

Methods: Primary cultures of keloid and adjacent normal dermal fibroblasts (n = 4) as well as frozen and paraffin-embedded keloid and normal dermal tissues (n = 12 and n = 17 respectively) were examined by Western blot and immunohistochemical analyses for the expressions of Cdc25A, Cdc25B, Cdc25C and phosphorylated Cdk2.

Results: Cdc25A protein levels were frequently increased in keloid fibroblasts as compared to the adjacent normal-appearing fibroblasts or different normal dermal fibroblasts. In contrast, Cdc25B and Cdc25C were none or rarely expressed. The increased levels of Cdc25A were associated with lower levels of its substrate, Cdk2, in a phosphorylated or inactive form.

Conclusions: Taken together, our data show that Cdc25A protein levels increase in keloid fibroblasts and this increase may be sufficient to activate its substrate, Cdk2, and accelerate the cell cycling. These results may have implication for the development of strategies to silence Cdc25A activity after the wound healing as a therapeutic modality for the keloid lesions.

S-II-02

THE EFFECTS OF IL-10 AND TNF α ON WOUND HEALING OF INTESTINAL ANASTOMOSIS

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Aim: This study aimed to define the participation of local expression of IL-10 and TNF α around the anastomotic segment and clarify the effects of IL-10 and TNF α on anastomotic wound healing after digestive surgery under septic condition.

Methods: Experimental animals were divided into LPS and control groups, which had either LPS or normal saline solution injected into the peritoneal cavity 24 h before transection and anastomosis of the colon. Immunohistochemical staining for IL-10 and TNF α on tissue samples were examined after the operation. Fibroblasts were cultured with IL-10 and TNF α , then proliferation rates were determined using the MTT assay. Type I collagen protein and MMP-I were detected by indirect immunofluorescence.

Results: In the LPS group, IL-10 and TNF α expression were more enhanced than that in the control group 24 h after the operation. IL-10 reduced fibroblasts proliferation in dose dependent fashion in the presence of TNF α . Indirect immunofluorescence showed that IL-10 reduced type I collagen protein and increased MMP-I.

Conclusions: Local expression of IL-10 and TNF α at the anastomotic site acted as an inhibitory factor in the wound healing process. IL-10 suppressed the remodeling of the extracellular matrix during wound healing.

Symposium II Gastrointestinal Anastomosis*Moderated by Kazuo Shirouzu*

S-II-01

DYNAMICS OF MYENTERIC NERVES AFTER TRANSECTION OF THE MUSCLE COAT OF THE RAT SMALL INTESTINE; THE INVOLVEMENT OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) AND ITS RECEPTOR (RET) IN THEIR GROWTH

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The present immunohistochemical study was designed to investigate the manner of the myenteric nerve regeneration and expression of glial cell line-derived neurotrophic factor (GDNF) and its signaling receptor (Ret) in nerves after transection of the muscle coat in the rat small intestine. The enteric neurons and enteroglia cells were immunohistochemically determined using antibodies against protein gene product 9.5 and S-100 protein, respectively. The neuronal sprouts issued from the severed nerve stumps 12 h after the operation, and thereafter extended into the lesion. The proximal portions of outgrowing neuronal fibers were gradually enveloped by regenerating enteroglia cells to become thick nerve bundles. The regrowing neurons and their associated enteroglia cells developed into an irregular network in the myectomized area on postoperative day 5. The elongation of the regrowing nerves was conspicuously accelerated from postoperative day 3 in accordance with the regrowing neurons started to associate with the enteroglia cells. Under normal conditions, faint immunoreactivity for GDNF and Ret was selectively localized in the enteroglia cells and neurons within the myenteric ganglia, respectively. Following myectomy, the both immunoreactivities were significantly intensified in the nerve stumps and ganglia proximal to the lesion. The regenerating enteroglia cells closely associated with the regrowing neurons in the lesion exhibiting a dense immunoreaction to GDNF, whereas the neuronal fibers were richly supplied with reaction products of Ret in their entire course. The present findings suggest that the enteroglia cells, contacting with regrowing neurons, may promote the myenteric nerve regeneration in the rat small intestine, via the GDNF-Ret signaling system.

S-II-03

A STUDY OF WOUND HEALING IN CHRONIC HEMODIALYSIS PATIENTS AFTER ABDOMINAL OPERATION

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Aim: This paper reports on the delay in wound healing of chronic hemodialysis patients who under-went abdominal surgery.

Methods: The subjects were 19 hemodialysis patients who had had elective abdominal operations and 20 hemodialysis patients who had undergone emergency abdominal surgery. For all subjects, we retroactively investigated the incidence of wound complications and the differences in postoperative values for TP, ALB, RBC and HGB between the wound-complication group and the no-wound-complication group.

Results: In the elective-surgery group, the incidence of wound complications was 21%, including one suture failure, one wound deficiency and three wound infections. No patients died. In the emergency-surgery group, the incidence of wound complications was 40%, with two suture failures, four wound deficiencies and two wound infections. The postoperative death rate was 20%. There was no significant difference in TP, ALB, RBC, or HGB between the wound-complication group and the non-complication group in either the elective or the emergency surgery group.

Conclusions: In hemodialysis patients, pre- and postoperative infection had a great influence on wound healing.

Symposium III Stoma and its Management

S-III-01

A CASE OF SKIN TROUBLE AROUND THE ILEOSTOMY IMPROVED BY APPLICATION OF BFGF SPRAY

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The content of the effluent from an ileostomy is high-volume, liquid, and contains proteolytic enzymes. It is crucial to maintain a stoma appliance that protects the surrounding skin. This is an interesting report of a 74-year-old female who present bladder carcinoma and underwent total cystectomy, ileal conduit, low anterior resection of the rectum and loop ileostomy for covering the anastomosis at July 7, 2002. After surgery, the stoma was depressed in fatty abdomen and resulted in the trouble surrounding skin. The erosion around stoma was not healed easily. We applied bFGF spray to the erosion around the ileostomy. One week later, an ostomy appliance was able to apply longer, because of reducing exudates from the erosion.

The difficulty to maintain the good status surrounding skin with ileostomy was higher up with increasing meal and activities of diary living. The application of bFGF spray advanced the healing skin erosion. It is useful for caring the skin trouble with ileostomy.

S-III-02

CONSTRUCTION AND MANAGEMENT OF LOOP ILEOSTOMY

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Aim: The aim of this study was to determine the morbidity associated with both construction and reversal of loop ileostomies.

Methods: Thirty-three patients who had loop ileostomies constructed and 31 of these same patients who had their loop ileostomies reversed between 1994 and 2001 were reviewed.

The loop ileostomies were constructed in round-shaped (2.5~3 cm in diameter) like a end ileostomy and everted to produce a 2~3 cm stoma without a rod

Results: The average size of ileostomies was 28×29×25(height)mm. Five patients had complications arising from ileostomy construction including 4 parastomal irritation, 1 ileus, and 1 stomal ulcer. All complications improved with conservative management. Mean time to ileostomy reversal was 78 days. Of 31 patients who had loop ileostomies reversed, 10 patients had staple closure (functional end-to-end anastomosis with ENDO GIA 45). Nine patients had complications associated with reversal including 6 small bowel obstruction due to anastomotic stenosis(anastomotic edema), 1 leakage and 2 SIRS. Of these 9 patients, 2 required surgical intervention. One patient suffered leakage required reanastomosis, one for SIRS required reconstruction of loop ileostomy. Of 6 patients developed small bowel obstruction, 3 required long intestinal tube decompression. All patients improved with conservative management. 10 patients who had their loop ileostomies closed by functional end-to-end anastomosis had no bowel obstruction and other complications.

Conclusions: Defunctioning loop ileostomy is associated with low morbidity. We recommend a defunctioning ileostomy as the procedure of choice for temporary fecal diversion, and functional end-to-end anastomosis in reversal of ileostomies.

S-III-03

LONG-TERM CLINICAL OUTCOMES OF A NEW COLOSTOMY TECHNIQUE

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Aim: Quality of Life (QOL) of patients after cancer surgery has become more important as success rate of surgical treatment has been improved. Anal sphincter preservation has been concerned in treatment for colon cancer, in particular rectum cancer. However, in order for patients to become cancer free, colostomy is the essential treatment. In usual stoma, skin contacts directly with colon membrane, and this sometimes induces (i) skin troubles around the stoma (e.g., ulcer, abscess, fistula), and (ii) constriction, collapse, perforation, hernia or obstruction of the colon. The skin troubles are the complications that are unavoidable only through the improvement and control of the equipment because the skin directly touches to the stool. Therefore, preventive measures should be employed at colostomy. For this purpose, a new colostomy technique was applied from the point of plastic and reconstruction surgery.

Methods: In order to reduce the duration and area of direct contact with stool, local flap and split-thickness skin grafting are applied. Colon is pull out to the abdominal wall by using the usual surgical procedure. The stoma is kept at a certain height from the abdominal wall by using the flap and the graft.

Results: To date, the longest follow-up period is 8 years and 7 months. Frequency of skin problems due to direct contact with stool was reduced, infection and constriction on and around the stoma have been prevented, and defecation became able to be better managed.

Symposium IV Hybrid Type Tissue Regeneration

Moderated by Norio Kumagai

S-IV-02

C2C12 CELLS DIFFERENTE IN THREE DIMENSIONAL CULTURE IN COLLAGEN GEL

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Aim: The movements of the cell population are different between 2-D culture and 3-D culture. From the observation of morphology of skeletal muscle cell 3-D culture, We expect that skeletal muscle cells differentiation is accelerate in the collagen gel 3-D culture, and the proliferation is suppressed. The purpose of this study is to investigate the difference between 2-D culture and 3-D culture of C2C12 cells.

Methods: C2C12 skeletal muscle cells are incubated following three difference conditions for 48 hours, plastic dish 2-D culture, collagen coated dish 2-D culture and collagen gel 3-D culture. The culture medium is Dulbecco's modified Eagle medium containing 10% fetal bovine serum and 1% penicillin/streptomycin. Collagens are removed by collagenase treatment and cells are homogenized. After centrifugation the top clear layer is used for CPK assay and protein development analysis by Western blotting

Results: After 48 hour incubation, we observed cell morphology by a phase contract microscope. Cell fusion was observed in collagen gel 3-D culture. The fusion cells have many nucleus in the cytoplasm called synthetium. But in plastic dish 2-D culture and in collagen coated dish 2-D culture synthetiums were not observed and cells were mononuclear and monolayer. Cell proliferation was suppressed in collagen gel 3-D culture. CPK activity was five times activated in collagen gel 3-D culture than in plastic dish 2-D culture.

Conclusions: We suggest skeletal muscle cells C2C12 are activate differentiation by collagen gel 3-D culture.

S-IV-03

ECTOPIC BONE FORMATION ACCELERATED BY HUMAN MESENCHYMAL STEM CELLS AND OSTEOGENIC CYTOKINES VIA NUTRIENT VESSEL INJECTION IN NUDE RAT

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Critically larger bone and skin defects often lack the enough nutrient blood supply to induce normal wound healing. Additionally, deteriorated environment such as poor vascularity due to the hard fibrosis after irradiation or extensive tissue loss due to the highly damaged tissue or severe bacterial contamination, leads to finding another donor sites or another methods to repair. Potential free vascularized flaps are widely accepted for reconstruction purpose, however, the donor-site morbidity is sometimes concerned and its clinical application may be limited. Tissue engineered tissues are now most actively investigated and partially clinically appropriate for use. In order to assist the bone wound healing, the human mesenchymal stem cells (hMSCs), wrapped with the abdominal fascial flap, which is nutrient of superficial epigastric vascular systems in bone, and the full-thickness dorsal defect covered by artificial skin substitute as a carrier in skin, are used as cell source with potential differentiation with specific cytokines in a nude rat model for eliminating T cell immunity. The hMSCs and osteogenic cytokines such as bone morphogenetic protein-2 (BMP-2) and basic fibroblast growth factor (bFGF) were intra-arterially injected and incubated for 10 minutes and then the gelatin-carrier were wrapped with the abdominal superficial fascia and investigated for the subsequent experiments. The heterotopic bone formation was most significantly observed in 4 weeks after injection and this may be used clinically to enhance the compromised bone healing.

S-IV-04

A VASCULARIZED ARTIFICIAL BONE GRAFT USING THE PERIOSTEAL FLAP AND POROUS HYDROXYAPATITE; BASIC RESEARCH AND PRELIMINARY CLINICAL APPLICATION

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Aim: Vascularized periosteum has an ability of new bone formation, and porous hydroxyapatite (HA) has a property of bone conduction. A basic research was performed to investigate the possibility of making a "hybrid" vascularized artificial bone from porous HA integrated with the vascularized periosteum.

Materials & methods: A latissimus dorsi musculoperiosteal flap was raised in rabbit. In one group, HA blocks (porosity; 55%, dimension; 8×4×2mm) were ligated to the cambium layer of periosteum and muscle, and in the other group HA blocks combined with rhBMP-2 were applied in the same manner. HA blocks were removed 4, 8 and 12 weeks after the operation and were examined histologically. This method was applied preliminarily to 5 patients who needed bony repair of anterior chest, midface, and mandible.

Results: Osteogenesis was observed within the pores of the blocks over the course of time. In rhBMP-2 group, osteogenesis progressed rapidly to the same extent as in without rhBMP-2 group. In clinical cases, postoperative bone scintigraphy demonstrated radioactive uptake into HA implants, and biopsy revealed bone neogenesis into the pores of HA.

Conclusions: Our basic research and preliminary clinical applications suggested that the possibility of making the hybrid type of vascularized artificial bone preparation with HA using this method. This will enable less invasive procedure and custom-made reconstruction without the need of vascularized autogenous bone grafts.

S-IV-05

STUDY IN HYBRIDIZED TISSUE ENGINEERED TRACHEA BY USING ACELLULAR TRACHEA SCAFFOLD

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Aim: A method for preparing acellular trachea scaffold and its effectiveness were investigated by using rabbits and dogs

Methods: The sacrificed dog or rabbit trachea was collected. The spiral stent of stainless steel was inserted in the obtained trachea. To remove all of cellular components in tissues, the trachea was rinsed with sterilized 0.5% Triton X-100 for 24 to 48 hours at ambient temperature and then for removing the detergents completely using fresh water. The acellular trachea obtained was lyophilized and sterilized by ethylene oxide gas. Before implantation, the lyophilized acellular trachea was soaked in phosphate buffered saline containing 0.5% gelatin and other adhesive molecules for 2 to 18 hours at 37 degree. After 15mm of rabbit neck trachea was removed surgically under anesthesia, the same length of reengorged acellular rabbit trachea was implanted the removed region. In the case of dog, 50mm of thoracic trachea was removed under the mechanical ventilation and then the same length of reengorged acellular trachea was implanted by technique of end to end anastomosis. The implanted trachea was rapped by omentum. The effectiveness of acellular scaffold on implanted-animals was evaluated by endoscope finding.

Results: 1) The rabbits implanted reengorged-acellular trachea survived for minimum 10 days and maximum 60 days. It was suggested that the cause of death was the infection of implantation region. 2) The dog implanted reengorged-acellular trachea survived for over 60 days at least. The cause of death was strangulated hernia. 3) The acellular trachea containing various growth factors or cultured with fibroblasts was not always effective.

Discussion: It is not necessary for the animal implanted acellular trachea to be administered the immunodepressants such as the animal implanted cryopreserved trachea. From these results, the tissue engineered acellular trachea may be more effective than the cryopreserved trachea.

Panel Discussion I Treatment of ASO, Non-Healing Ulcer

Moderated by Takehisa Iwai, Motohiro Nozaki

P-I-01

TREATMENT OF SKIN DEFECTS AND SKIN ULCERS WITH BFGF PREPARATION

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Abstract: On 11 patients with skin defect and refractory ulcer, treatment was conducted using bFGF preparations. Good granulation, epithelialization and reduction of ulcer were observed in all patients by once-daily spraying of this product after washing the topical region. This preparation is a drug developed aiming at the basic fibroblast-proliferating factor playing an important role in the wound healing process in the body and expecting the therapeutic effects on refractory skin ulcer. Although there are some restrictions including the tropical conditions such as infection and adherence of necrosed tissues and careful administration to the patients with a past history of malignant tumor, its good therapeutic effects were expected in the patients ineffective with other drugs, and this product was evaluated to be useful from the viewpoint of QOL, such as shortening of treatment period, reduction of cost and low invasion.

P-I-04

TREATMENT FOR LIMB ULCER WITH SEVERE ISCHEMIA: THERAPEUTIC ANGIOGENESIS BY AUTOLOGOUS TRANSPLANTATION OF BONE-MARROW

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Aim: Limb ulcer due to severe ischemia often needs revascularization. In our hospital, the result of lower extremity bypass surgery for these patients is satisfactory, however, indication of this procedure is limited pathologically and technically. We recently have demonstrated a possibility of efficacy of therapeutic angiogenesis by autologous bone marrow cell transplantation for severe ischemic ulcer of extremity.

Methods: Eight patients (9limbs; 3limbs with arteriosclerosis obliterans, 6limbs with Buerger's disease) with ischemic ulcer of lower and/or upper extremity were treated with implantation of autologous bone marrow mononuclear cell into ischemic skeletal muscles. Four weeks after transplantation, efficacy of this procedure were evaluated by Visual Ana log Scale, angiography and Laser Doppler Flow Analysis (LDFA).

Results: In 4 out of 9 limbs, the ulcers healed dramatically. Seven limbs revealed angiographically improvement and increase of blood flow by LDFA.

Conclusions: Our findings suggest that therapeutic angiogenesis by autologous bone marrow cell transplantation is an effective and safety strategy for patient with peripheral arterial disease. We considered that it is more effective to Buerger's disease than arteriosclerosis obliterans.

Reference: I.Shintani S, Murohara T, *et al*; Augumentation of postnatal neovascularization with autologous bone marrow transplantation. *Circulation* 2001; 103:897-95.

P-I-05

FLAP REPAIRS FOR INCURABLE LOWER LEG ULCERS

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Incurable ulcer in the lower legs means avascular skin defects exposing bone and osteomyelitis which cannot be repaired even with skin grafting. A total of 151 patients with incurable ulcers were operated with flaps; 60 cases of traumatized avascular defects, 20 diabetic ulcers, 17 osteomyelitis, 14 malignant tumors, 5 arterial obstructions, and 5 arteriovenous malformations, and others. A total of 61 island flaps were used; 20 posterior tibial perforator flaps, 7 saphenous flaps, 7 peroneal flaps, 4 anterior tibial flaps, 4 malleolar perforator flaps, 4 medialis pedis flaps, and others. In addition, a total of 82 free flaps using microvascular anastomosis were used; 24 flow-through anterior thigh flaps, 13 flow-through thoracodorsal artery perforator flaps (or latissimus dorsi MC flap), 8 paraumbilical (or deep inferior epigastric artery) perforator flap, 6 saphenous venous flaps, 7 combined flaps, and 24 others. In conclusions, small ulcers could be repaired with minimal invasive methods including local perforator flaps and small muscle flaps under local anesthesia. Free flow-through flaps and free bypass flaps (for diabetic gangrene with ASO), and combined osteocutaneous flaps (for massive segmental defects after resecting advanced carcinoma) are indicated for large ischemic defects.

Panel Discussion II Clinical Application of Growth Factor

Moderated by Ichiro Ono, Takenori Ochiai

P-II-01

DEVELOPMENT OF ALLOGENEIC CULTURED DERMAL SUBSTITUTE (CDS)

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The authors have developed a CDS by culturing fibroblasts on the two-layered spongy matrix of hyaluronic acid (HA) and atelo-collagen (Col). This CDS is designed to promote wound healing by synergistic effect of fibroblasts and matrix. Both HA and Col molecules seem to function biologically in the process of wound healing. HA molecules play a critical role in several cellular functions such as migration and proliferation by promoting adhesion and disadhesion between the cell and the tissue substrate. Besides providing structural support and strength to the new tissue, Col molecules have a profound effect on the cells within and on its matrix. Col and Col-derived peptides act as chemoattractants for fibroblasts in vitro and may have a similar activity in vivo. Fibroblasts seeded on the Col surface of two-layered spongy matrix were found to attach, proliferate, and release vascular endothelial growth factor (VEGF) as well as fibronectin. The cryopreserved CDS was found to keep the original potency to release VEGF after thawing followed by re-culturing. Multi-center's clinical research using allogeneic CDS has been proceeded as a national millennium project for regenerative medicine. These products are able to be stored in a freezer and transported to other hospitals in a frozen state. The clinical evaluation involving 180 cases has been already conducted using allogeneic cryopreserved CDS at 30 hospitals across Japan since April 2001. The results obtained in our clinical study suggest that this type of allogeneic CDS is able to provide an effective therapy for patients with severe full-thickness skin defects. These excellent clinical evaluations seem to be closely related to the results obtained in this fundamental study, especially related to the potency of cryopreserved allogeneic CDS to release VEGF and fibronectin.

P-II-02

LEUKEMIA INHIBITORY FACTOR GENE AND VASCULAR ENDOTHELIAL GROWTH FACTOR PROTEIN CAN MODULE EMBRYONIC FIBROBLASTIC DIFFERENTIATION VIA GP130-STAT AND MAPK SIGNAL TRANSDUCTION PATHWAYS

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The combined application of cytokines on embryonic fibroblasts and dermal substitute were extensively studied for optimal skin defect coverage. Signalling of combined treatment of leukemia inhibitory factor (LIF) and vascular endothelial factor (VEGF) were elucidated and subsequently the in vivo applications of both were tested in an artificial dermal substitute. Mouse embryonic fibroblast cells, BALB-3T3, were stably transfected with mouse full length LIF cDNA and added to various doses of VEGF for detection of signalling interaction. LIF-transfected cells and VEGF treatment were tested with pig-tendon derived collagen dermal substitute in the backs of BALB/c male mice for 14 days. LIF-transfected cells as well as vector-transfected fibroblasts significantly proliferated by 1, 10, or 100 nM VEGF on days 3 and 5. LIF-transfected cells showed rapid phosphorylation of STAT 3 from 1 minute to 60 minutes after VEGF treatment, while vector-transfected cells failed to induce such phosphorylation after VEGF treatment. Erk MAP kinase phosphorylation was observed from 1 to 15 minutes in LIF-transfected and 10 nM of VEGF and 1 to 30 minutes in LIF-transfected and 100 nM VEGF treatments. In in vivo analyses, LIF-transfected embryonic fibroblasts with 50 µg of VEGF markedly enhanced collagen I expression and CD 34 angiogenic marker on days 7 and 14. LIF transfection induced constitutive STAT signaling and enhanced phosphorylated-Erk MAP kinase with exogenous VEGF. In vivo study revealed that the combined application of LIF-transfection of embryonic fibroblasts with an angiogenic factor such as VEGF in the template of an artificial dermis.

P-II-03

EFFECT OF PLATELET DERIVED WOUND HEALING FACTOR (PDWHF) ON THE ANGIOGENESIS IN THE CASES OF ARTIFICIAL DERMIS GRAFTS

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Aim: The effect of platelet derived wound healing factor (PDWHF) with or without cultured cells on angiogenesis in the cases of artificial dermis grafts was investigated.

Methods: Wistar strain rats were used for this study. The PDWHF was prepared from platelets. The endothelial cells and fibroblasts were prepared from thoracic aorta and back skin respectively. Then 2 experimental models were designed. 1) Full-thickness wounds were created on back of the animals. Artificial dermis (TERUDERMIS[®], TERUMO Co, Japan) were grafted to the wounds. 2) Femoral artery and vein were isolated as a vascular pedicle and wrapped with a folded sheet of the AD (1×2 cm). Prior to AD grafts, the AD were treated with the PDWHF and/or cultured cells.

Results: The combination use of the PDWHF and cultured cells showed best angiogenesis in both experimental models.

Conclusions: The result of the present study revealed that treatment with PDWHF combined with cultured cells accelerate the wound angiogenesis.

P-II-04

EARLY PHASE REGULATION OF HUMAN MESENCHYMAL STEM CELLS AND THE UNIQUE PROFILE OF THE PROLIFERATION BY BONE MORPHOGENETIC PROTEIN-2

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Human mesenchymal stem cells (hMSCs) obtained from a single donor of the iliac crest, was further investigated for the cell proliferation, cell cycle profiles, gene expressions and the ultrastructures by electron microscopy. The hMSCs significantly increased the cell number by day 2 after by treatment of bone morphogenetic protein (BMP)-2 alone, or basic fibroblast growth factor (bFGF) alone or combination of both in the serum-free condition ($P < 0.01$). The hMSCs demonstrated the remarkable proliferation cell nuclear antigen notably at day 1 and pituitary tumor transforming gene all through the experiment, suggesting the cell cycle progression by BMP-2 treatment as well as the strong cellular nuclear BrdU expression by immunocytochemistry. The fluorescence-activated cell sorter also demonstrated the similar pattern of the cell cycle progression between BMP-2 treatment in the serum-free and 10 % fetal bovine serum treatment. The BMP-2 treated hMSCs demonstrated the heterochromatin in the nucleus, suggesting the cell differentiation and the well-developed granular endoplasmic reticulum, indicating the protein production. The hMSCs successfully proliferate, the cell cycle is progressed, and the cell ultrastructure morphology suggest the remarkable nuclear and of granular endoplasmic reticulum induction by BMP-2 treatment in the serum-free condition.

P-II-05

EFFECTS OF BMP-2 GENE AND bFGF PROTEIN COMBINED WITH POROUS HYDROXYAPATITE ON BONE FORMATION

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In this study, the bone defects created on animal cranium which treated by BMP gene (cDNA plasmid) and bFGF protein introduced with porous HAP and the bone formation was analyzed histopathologically. The bone defect of 1.2 cm in the diameter was made on rabbit's cranium and HAP pellets of exactly 1.0 cm in diameter were implanted after completion of hemostasis. The amount of the bone formation was compared with or without the presence of the BMP-2 cDNA plasmid. In addition, simultaneous administration of bFGF containing solution with or without BMP gene were performed to analyze the effect of combined use of them. The bone formation was vigorously observed in the cranial defect in the groups, which received the BMP-2 gene with HAP at three weeks after the operation, and complete ossification was observed at 9 weeks after the operation. In the HAP which containing BMP-2 gene implanted group, although it revealed that new bone formation was evident surrounding the HAP pellets at three weeks after the operation, the induced bone tissue did not fill to the entire pores of the HAP pellets even at 9 weeks after the operation which condition was improved by coadministration of bFGF with BMP gene. Those results show that at the BMP gene therapies the bone formation in the pores of the HAP pellet was promoted by coadministration of bFGF. It is possible the effects of the administration of the BMP-2 gene will be improved, from the above-mentioned result, through coadministration of bFGF and BMP gene.

Treatment of Keloid and Hypertrophic Scar

Moderated by Hiko Hyakusoku

001

TREATMENT OF HYPERTROPHIC SCAR WITH THE SILICONE CUSHION

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Aim: Various treatments for hypertrophic and keloid scars have been attempted including electron beam irradiation, local triamcinolone injection, oral tranilast administration, use of silicone sheets, and compression therapy using splints. Among them, we have been attempting silicone cushion patching on hypertrophic scar.

Methods: Twenty cases (10 males, 10 females) with hypertrophic scar were treated with silicone cushion. Scar surfaces were kept in contact with silicone cushion for as long periods of time as possible every day. Results were assessed in scores using objective findings (redness, bulging, induration) and subjective symptoms (itching, spontaneous pain, tenderness).

Results: As for objective findings, redness remained unchanged in one case, but bulging and induration were found improved in all cases. As for subjective symptoms, itching and tenderness were found improved in all cases and clinical course considered proving usefulness of the treatment was obtained.

Conclusions: Silicone cushions have highly viscous silicone oil enclosed in the silicone pack designed to generate negative charge electrostatic fields. Generation of adequate and sustaining electrostatic fields is important for useful clinical effect to exhibit on hypertrophic scar recession. Generation of negative charge electrostatic fields was confirmed on potential determination data with an electrostatic field meter as well.

003

GENE EXPRESSION PROFILES FOLLOWING ELECTRON IRRADIATION OF CULTURED KELOID AND NORMAL SKIN FIBROBLASTS BY CDNA MICROARRAY ANALYSIS

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Aim: When surgery with postoperative superficial electron irradiation is applied, the recurrence rate of keloid lesion has been found to be <30%. In this study, we assessed the molecular changes underlying the effect of electron irradiation by differential global gene expression analyses of both cultured keloid and normal skin fibroblasts.

Materials and Methods: Primary cultured fibroblasts from 4 active keloids and their adjacent normal dermal tissues were irradiated at a calibrated dosage of 15 Gy with 6 MeV electron beam generated by a linear accelerator. Corresponding paired non-irradiated cells were used as control. RNA isolated from the collected cells was labeled with ³³P, hybridized to the cDNA microarray gene filters and analyzed.

Results: After irradiation, the gene expression profiles of keloid and normal skin fibroblasts were closely similar. Electron irradiated keloid fibroblasts showed suppressed levels of collagen typeI (alpha2), collagen typeVI (alpha1), matrix metalloproteinase 2, fibronectin 1, insulin-like growth factor binding protein 3, alpha-1-antichymotrypsin and heparan glucosaminyl 1 as compared with their non-irradiated counterparts.

Conclusions: Downregulation of matrix synthesis and upregulation of protease inhibitors and apoptosis promoting genes by electron irradiation may inhibit keloid development. This mode of therapy appears to exert a positive effect toward lowering the recurrence rate of keloid formation.

004

POSTOPERATIVE ELECTRON-BEAM IRRADIATION-THIRTEEN YEARS' EXPERIENCE AND FUTURE PROSPECTS IN OUR HOSPITAL-

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Purpose and Methods: Between 1988 and 2000, 378 cases of keloids were treated and the therapeutic outcomes were evaluated. For this study, 147 keloids in 129 patients were selected.

Results: The overall recurrence rate was 32.7%. Analysis of the therapeutic outcomes showed that the recurrence rates in the sites with high stretch tension, such as the chest wall, and the scapular and suprapubic regions were statistically higher than in sites without high tension, such as the neck, earlobes and lower limbs ($p = 0.0017$).

Discussion: The results suggested that keloid sites with a high risk of recurrence should be treated with escalated radiation doses. However in our experience, pigmentation increases when the radiation dose is increased. Additionally, pigmentation can be suppressed by the following methods: 1. reducing the one-time dose of irradiation while keeping the total dose unchanged; 2. lengthening the irradiation interval. Therefore we made a new protocols and provide further insights into the treatment: Total 20 Gy (4 days): Anterior chest wall, Scapular region and Suprapubic region; Total 10 Gy (2 days): Ear lobe; Total 15 Gy (3 days): Others.

Cytokine

Moderated by Tatsuo Nakajima

005

ACCELERATION OF WOUND HEALING IN HEALING-IMPAIRED *DB/DB* MICE WITH A PHOTOCLOSSLINKABLE CHITOSAN HYDROGEL CONTAINING FIBROBLAST GROWTH FACTOR-2

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Aim: The purpose of the present study has been to assess possibilities of the FGF-2 incorporated chitosan hydrogel as a dressing for wound occlusion and healing acceleration in healing-impaired *db/db* mice.

Methods: Mutant diabetic mice, C57BL/KsJ *db/db*, and their normal littermates (*db/+*) were used in this study. After full thickness-round wound (about 100 mm²) were prepared on the back of each mouse, the FGF-2 containing chitosan hydrogel was added onto the wound of each mouse and was irradiated with UV light. Similar full thickness-round wound were also prepared as controls without any treatment. The changes in wound area of mice were measured. The skin including the wound was removed from each mouse for histological examination.

Results: For the *db/db* mice treated with the FGF-2-incorporated chitosan hydrogel, healing was faster than only chitosan hydrogel-treated and control wounds. On the other hand, the incorporated FGF-2 in the chitosan hydrogel did not show a stimulatory effect on the wound healing of *db/+* mice. The vascularity of wound areas of *db/db* mice on day 4, evaluated immunohistochemically with anti-murine CD34, was markedly increased in FGF-2-incorporated chitosan hydrogel-treated wound.

Conclusion: FGF-2-incorporated chitosan hydrogel may become accepted as an occlusive dressing for healing impaired wound management.

007

DIFFERENCES IN EXPRESSION OF BASIC FIBROBLAST GROWTH FACTOR DURING WOUND HEALING BETWEEN ORAL MUCOSA AND SKIN

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Introduction: Oral mucosa heals faster with less scar than skin, but its mechanism has not been elucidated enough. We have studied whether there are any differences in the dynamic study of the bFGF expression in vivo during wound healing between oral mucosa and skin.

Methods: Male Wistar rats were anesthetized, and a 3mm circular excisional wound was made on the dorsum skin and intrabuccal mucosa of each rat. At days 0,1,3,5,7 and 10 post-wounding, wounds were harvested, and embedded in OCT. From photographs of the wounds, each wound area was measured by means of NIH images. Also, for histological study, sections were stained haematoxylin and eosin, and for immunohistochemical study, sections were stained with anti-bFGF antibody.

Results: Oral wounds contracted significantly faster than skin. Regenerated skin appeared at day 1 post-wounding in oral wounds, but at day 3 in skin. Re-epithelialization was completed on day 5 post-wounding in oral, but on day 7 in skin. The number of bFGF-positive cells gradually increased in the granulation tissue, and the peak of positive cells in oral was observed on day 5 post-wounding, while that in skin on day 7.

Conclusion: Oral mucosa healed faster than skin. The peak of bFGF expression was faster in oral mucosa than skin. It was thought that bFGF effects the differences during wound healing between oral mucosa and skin.

008

THE EFFECT OF THE GELATIN SHEET CONTAINING bFGF ON WOUND HEALING

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Aim: It is well recognized that bFGF accelerates proliferation of almost all cells concerned with wound healing and there is a report that bFGF was well sorbed with time to the acidic gelatin hydrogel with isoelectric points of 5.0. We investigated the effect of the acidic gelatin sheet containing bFGF.

Methods: Full thickness defects of skin (1.5×1.5 cm) were created on the backs of mice. 1) The wounds were covered with gelatin sheets (2×2 cm) containing bFGF (100 µg/site), (A), and without bFGF (B). The concentration of bFGF in plasma was estimated by ELISA. 2) The wounds were covered with A, B and hydrogel dressing (control group, C) and wound area was measured with computer planimeter and neopeithelium was observed using the light microscope.

Results: 1) The concentration of bFGF in plasma in (A) was statistically greater than (B) by the 7th day. 2) Statistically smaller wound area was found 2 weeks postoperatively in (A) than in (C) and (D). Neopeithelium from edge of the wound was statistically longer in (A) than in (C).

Conclusions: Controlled release of bFGF from the acidic gelatin sheet was found and acidic gelatin sheet containing bFGF promoted neopeithelization and wound closure. The acidic gelatin sheet containing bFGF was thought to be effective on wound healing.

009

CALCIUM-ZINC ALGINATE AND RECOMBINANT HUMAN bFGF; ITS EFFICACY FROM THE HISTOLOGICAL STANDPOINT

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Aim: To evaluate the efficacy of wound dressing material, Calcium-Zinc Alginate: Curasorb Zn[®] as a carrier of the recombinant human bFGF, histological detection was performed.

Methods: Full thickness skin defect on the back of Wistar rat was made for four groups; the control group, bFGF group; topical application of recombinant human bFGF for 5 µg per day, Ca-Zn Alginate group; topical use of the dressing and the group that both the bFGF and Ca-Zn Alginate were applied, nine rats for respective group.

Results: Significant granulation was noted both bFGF group and Ca-Zn Alginate group compared to the control group. Fibrosis was noted in the control and Ca-Zn Alginate group whereas no fibrosis was noted in the bFGF group. Numerous amounts of cells were noted in the bFGF group which indicate strong inflammation. Also, marked abscess formation was noted in the bFGF group. The greatest thickness of granulation was obtained in the group that both the bFGF and Ca-Zn Alginate were applied.

Conclusions: It was considered that the appropriate use of the carrier for the bFGF is important to control infection. The Ca-Zn Alginate dressing was effective to suppress abscess formation.

011

EFFECTS OF GRANULOCYTE COLONY-STIMULATING FACTOR IN RATS WITH SEVERE ACUTE PANCREATITISHongFang Tuo, Masanobu Nakashima, Nobutsugu Abe, Masanori Sugiyama, Yutaka Atomi
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Aim: It is reported that Granulocyte colony-stimulating factor (G-CSF) increases the number and functions of the neutrophils of blood in animal models. In the present study, we observed the effects of G-CSF in rats with severe acute pancreatitis.

Methods: Pancreatitis was induced by injection of 0.2 ml of 3% taurocholate acid into biliopancreatic duct of the Wistar rat. Thirty rats were randomized into three groups. 1: Control group (C group). 2: Acute pancreatitis (AP) group. 3: AP + G-CSF groups. G-CSF was administered via jugular veins 1 hour before induction of pancreatitis. Blood and ascites at 1 and 3 hours after induction of pancreatitis were measured for the number of neutrophils, their phagocytosis and bactericidal activity and the concentrations of TNF- α , IL-6 and IL-1 α .

Results: The phagocytic neutrophils increased in AP + G-CSF group ($22 \pm 4.3 \times 10^5$) compared with AP group ($10 \pm 1.9 \times 10^5$) ($p < 0.05$); the bactericidal neutrophils increased in AP + G-CSF group ($60 \pm 14.5 \times 10^5$) compared with AP group ($20 \pm 6.1 \times 10^5$) ($p < 0.05$). G-CSF did not increase any the concentration of TNF- α , IL-6 and IL-1 α in blood and ascites.

Conclusions: G-CSF increases the number of phagocytic and bactericidal neutrophils of blood and ascites in SAP rat, without increasing the concentration of TNF- α , IL-6 and IL-1 α , therefore improves host defense against infection.

Clinical Cases*Moderated by Yuiou Hata*

013

A NEW METHOD OF CLOSING THE STERNUM WITH ABSORBABLE SUTURE AND ABSORBABLE FIXED PINS

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Aim: In case of using metal wire in closing after the median sternotomy, several disadvantages have been reported, including long lasting subject's sense of incongruity, risks of tear of the wire and cutting to be made by the wire, tenderness and becoming an obstacle for the use of CT or MRI. In our facilities, in order to solve these problems, absorbable suture and absorbable rib fixing pins have been used in closing the sternum.

Methods: Objects of the median sternotomy we've had since October, 2001, are six cases. The suture used was 6 PDS codes of 1 mm in diameter and it went through the sternum and ligated it. Three absorbable pins of poly-L-lactide were used.

Results: A progress observation period after the operation is from one month to fourteen months. The prognosis have been good and complications not recognized. **Conclusions:** The way of closing the sternum with absorbing suture and absorbing rib fixing pins will make it possible to solve problems incurred from the metal wire suturing.

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015

THREE CASES OF PRESSURE ULCER CURED BY CONCOMITANT USE OF bFGF AND DRESSING

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Introduction: It is reported on 3 cases of pressure ulcer which were hard to cure surgically by flap plastic operation or else, were completely healed by concomitant use of the basic fibroblast growth factor (bFGF) and the dressing.

Case report: CASE 1:A 55-year-old male with a pressure ulcer 3 cm in diameter formed on the buttock. After excising the margin and spraying bFGF, the wound was covered with a dressing made of hydrofiber. It was completely cured in 3 months. CASE 2:A 65-year-old male with a pressure ulcer on the sacral region 30 cm the major axis. The pocket was cut open, and bFGF was sprayed inside. Amounts of the dressing material depended on volumes of the exudate. It was cured completely in 6 months. CASE 3:A 85-year-old male with a pressure ulcer 10 cm in the major axis on the greater trochanteric region of the right side. The margin was excised to open the pocket, bFGF was sprayed, and hydrogel was used as the dressing. It was cured in 6 months completely.

Discussion: Usefulness of the conservative treatment is reported based on the experience referring to these 3 cases with pressure ulcer, which were completely cured upon concomitant use of bFGF and the dressing material suitable to the conditions of the wound after thorough surgical debridement and washings.

016

IS THIS ULCER A TRUE "CHRONIC" ULCER?

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Aim: Lethal diseases occasionally present with a "chronic" ulcer. If a physician can not realize this fact, the ulcer might give rise to a misery result. We present three educational cases of chronic ulcers that give caution to physicians.

Case report: The first case was a hand ulcer due to postradiation squamous cell carcinoma, which eventually led to amputation of index and middle finger. The second case was a leg ulcer due to postburn squamous cell carcinoma, resulting in death caused by visceral metastasis. Both cases were treated as simple chronic ulcers at other clinics for a long time. The third case was a lower leg ulcer caused by infection with *cryptococcus neoformans*. The ulcer was covered with a split thickness skin graft after administration of anti-fungal drugs.

Conclusions: As seen in these cases, we must always have serious doubts why the ulcer is not easily healed. If the causative factor is not easily found, biopsies from the ulcer would be helpful for the true diagnosis of the ulcerative lesions.

Wound Management

Moderated by Yoshihide Otani

017

AN INVESTIGATION INTO BACTERIA OF INTRAPERITONEAL WASHED FLUID ON SURGICAL SITE INFECTION

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Aim: Surgical Site Infection (SSI) has been considered to be caused by the sticking bacteria on the surgical site. We have investigated how the sticking bacteria may influence the SSI in clean-contaminated surgery.

Methods: In clean-contaminated operations with a Protractor we washed intraperitoneal space of 26 patients, 14 male and 12 female, whose mean age were 67.2 ± 7.6 years old, with 3L of saline for the operations of upper gastrointestinal tract and 5L for under. We collected the washed fluid at pre-operation and post-operation, and abdominal fat for the culture of the sticking bacteria in those specimens.

Results: All SSI rate was 3.8%. Each detective rates of bacteria were 38.5%, 50%, and 46.2%, but each SSI rates were all 0%. We used the Protractor which may protect the sticking of bacteria on surgical sites, however many bacteria have been detected on them. There was only one patient who had been resulted in SSI. In this case the patient was suffered from hypovolemic shock in pancreatic-tail resection, however we could not detect any sticking bacteria on the surgical site.

Conclusions: Sticking of bacteria on surgical sites is not a chief cause of SSIs in clean-contaminated surgery. We guess there may be another cause as the bacterial-translocation for the SSIs in clean-contaminated surgery.

018

A CASE REPORT: SUCCESSFUL WET TREATMENT OF A DISSOCIATED AND INFECTED SURGICAL WOUND AFTER RADICAL VULVECTOMY

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A 72-year-old female, who had slight impaired glucose tolerance, was diagnosed the second stage of carcinoma of the vulva. On the second day after radical vulvectomy with inguinal lymphadenectomy, the center of the wound changed color into deep purple. On the 8th postoperative days, the swelling of the wound extended over the mons pubis and a thigh, so the infected wound, which reached the epimysium, was opened for the debridement. *Pseudomonas aeruginosa* and gram negative bacilli were detected from the wound. Firstly, the wound was cleaned by a physiological salt solution, streptokinase, and sulfadiazine silver. The gynecologist and nurses in charge discussed about the therapy under the consultation of a dermatologist and treated the wound by the wet treatment that a piece of gauze moisten by a physiological salt solution was exchanged 3 or 4 times a day after disinfecting by povidone iodine. On the 13th postoperative days, the infection was controlled and on the 19th days the skin was grafted from a thigh. After the successful skin grafting the patient was discharged. Carcinoma of the vulva tends to occur in elder women. Wounds are often dissociated because of an interruption in the circulation by the excessive extension of the skin. From the successful experience of wet treatment after a surgical wound infection, we recognized the necessity of close observation on surgical site and the importance of cooperation of the doctors and the nurses in charge.

019

2 CASES OF MEDIASTINITIS AFTER MEDIAN STERNOTOMY SURGERY TREATED WITH VACUUM-ASSISTED CLOSUREHideyuki Yanagi, Hiroto Terashi, Shinya Tahara
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Introduction: Mediastinitis, which is at times encountered after thoracic surgery with median sternotomy, is often resistant for treatments and in cases leads to death. We applied vacuum-assisted closure (V.A.C.) for 2 cases of post-sternotomy mediastinitis, in an original manner using readily available materials and made them healed. The details are reported.

Methods: We applied polyurethane foam (HydroSite[®], Smith & Nephew) with silicone tube (BLAKE[®], ETHICON) on the debrided wound, covered it with surgical draping film (Loban[®] 2, 3M Health Care), and applied continuous negative pressure with a wall suction. The dressing was changed at intervals of 2 to 3 days.

Results: (Case 1) A 53-years old male with complications of diabetes mellitus and hypertension underwent coronal artery bypass graft surgery for angina pectoris. Sternal infection occurred in postoperative period and the wound was opened. (Case 2) A 78-years old male underwent total aortic arch replacement surgery for aortic dissection. The wound was opened for a postoperative infection. We applied vacuum-assisted closure therapy for these 2 cases in the method presented above and observed promoted wound granulation and rapid contraction of wounds, with no manifestations of increasing infection or changes of circular conditions. Each of the cases was healed with vacuum-assisted closure in 7 Weeks without any surgical procedures.

Conclusions: We viewed vacuum-assisted closure therapy as another choice of the treatment for mediastinitis.

020

STUDY ON THE WOUND CURE AFTER LAPAROSCOPIC OPERATIONKenji Kakisako MD, Fumitaka Yoshizumi MD, Kimio Yamaguchi MD, Kazutoshi Kaketani MD,
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Aim: Regarding the recent change in the surgical technique of infraumbilical incision for laparoscope insertion from lateral incision to longitudinal incision to reach inside the umbilicus, we conducted this study to find the merits and demerits of change as well as to clarify the circumstance in which poor wound cure occurs after laparoscopic operation.

Methods: Total 608 patients who underwent laparoscopic operations between February 2000 and July 2002 at this hospital were used as the subjects of this study. Accordingly, the comparison was made between the 343 cases in the early stage (lateral incision) and the 380 cases in the late stage (longitudinal incision).

Results: Postoperative wound trouble occurred in 24 (3.9%) of the 608 cases. Infection occurred in the wound made by open laparoscopy in 5 patients. The trouble in the trocker insertion site itself was observed in 19 cases (3.1%). The infraumbilical wound trouble occurred in 10 cases in the early stage and 5 cases in late stage indicated, indicating lower trouble rate in the latter.

Cultured Skin • Deep Dermis*Moderated by Shigehiko Suzuki*

021

"EPITHELIZATION UNIT": A NEW CONCEPT IN SKIN WOUND HEALINGNaoto Yamamoto, Tomoharu Kiyosawa, Katsuyuki Arai
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Aim: Development of adequate connective tissue as a bed below new epidermis is important for successful epithelialization. However, how the dermal fibrous architecture newly forms and changes chronologically in that process is unknown. The aim of the present study was to determine the regeneration process of neodermis in skin wound healing from the viewpoint of neoformation of its fibrous architecture.

Methods: We investigated the repair process of an excised skin wound made on experimental animals with the use of alkali water cell-maceration scanning electron microscopy, which allowed a three-dimensional demonstration of individual collagen fibrils in their natural form and location.

Results: Both new papillary dermis and new epidermis underwent synchronous development in epithelialization, as if forming "a unit". The scar tissue has the same layered structure as seen in normal skin, namely epidermis including basement membrane, papillary dermis, and reticular dermis. The new epidermis on the new papillary dermis demonstrated a mature appearance and a mechanically stable condition.

Conclusions: The healing process proceeds as forming a unit composed by epidermis including basement and papillary dermis, and is finally completed with total coverage of the wound by this unit. We would like to call this unit, the "epithelialization unit".

022

PREINCUBATION REDUCES EPITHELIALIZATION PERIOD AFTER GRAFTING IN CELL-PRECOMFLUENT CULTURED SKINYasumi Saso, Takeshi Kawazoe, Shigehiko Suzuki, Kenji Tomihata*
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Purpose: We have developed a cultured skin with both epidermal and dermal components using two different types of collagen sponges as a scaffold. In addition, we succeeded in grafting cell-preconfluent cultured skin immediately after seeding the cells. In this study, we examined whether the incubation before grafting can reduce the period of epithelialization after grafting.

Method: We produced cultured skin as follow. We seeded fibroblasts on the dermal collagen sponge with a seeding density of 100,000 cells per square centimeter. After incubation for 4 hours, an epidermal collagen sponge was put on the dermal collagen sponge, and keratinocytes were seeded on the epidermal collagen sponge with a seeding density again of 100,000 cells per square centimeter. The cultured skin was incubated for 1, 2, and 3 days (n=3), and grafted them onto skin defects in SCID mice. Three, 5, 7, and 14 days after grafting, tissue specimens were harvested for histological and immunohistochemical examination.

Result: Three days after grafting, in 3 days incubation group, the cultured skins formed epithelium with a few layers, while in other groups, they did not. Five days after grafting, in 3 days incubation group, the cultured skins formed 6-8 epithelium layers, while in other groups, they did fewer layers. Seven days after grafting, in 3 days incubation group, the cultured skin formed epithelial layers consisting of cornified layers, and human type IV collagen was stained at the dermal-epidermal junction. In other groups, it was not stained. Fourteen days after grafting, all samples formed epithelial layers, and human type IV collagen was stained.

Conclusion: In 3 days incubation group, the epithelialization was completed earlier than that in the other groups.

023

DEVELOPMENT OF TISSUE ENGINEERING SKIN BASED ON ACELLULAR DERMAL MATRIX

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Aim: In tissue engineering of the skin, the selection of a scaffold or a matrix in which a cultured cells grow is quite important. We have developed a tissue engineering skin composed of human keratinocytes and fibroblasts on an acellular allogenic dermal matrix (ADM), derived from cryopreserved human skin.

Methods: ADM was prepared from cryopreserved split-thickness human skin by treating with Dispase and Triton X-100. The tissue engineering skin were produced by seeding human keratinocytes and fibroblasts on ADM. Several days after seeding, the tissue engineering skin was exposed to an air-liquid interface for another 7 days. Then, the histological structure of the skin and the production of growth factors by the skin were investigated.

Results: The produced ADM was found to be completely acellular with remaining structure of the basement membrane components, such as type IV collagen and laminin. The developed tissue engineering skin had stratified keratinocytes on the surface of the ADM migrating fibroblasts in the dermal collagen structure, resembling to the normal skin appearance. It was found that several important growth factors in wound healing process, such as TGF- α , TGF- β and VEGF, were produced by the tissue engineering skin.

Conclusions: It was suggested that ADM is suitable for a scaffold in tissue engineering of the skin.

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024

ACCELERATED EPITHELIZATION OF STSG DONOR WOUNDS BY CULTURED CELLULAR SHEET COMPOSED OF MIXTURE OF KERATINOCYTES AND FIBROBLASTS

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Aim: Available donor skin is so limited in extensive burns that wounds are usually closed using STSG harvested repeatedly from unburned skin. In such cases, it is important to accelerate the epithelization process of the donor wounds. We have developed a cultured cellular sheet composed of mixture of keratinocytes (KC) and fibroblasts (FB) seeded on a polyurethane membrane (mixed culture sheet) and investigated its effectiveness for the wound healing of STSG donor wounds.

Methods: Normal human KC and FB were primarily cultured from a surplus human skin in a skin graft surgery. After a few passages of the cultivation, both cells were seeded on a thin polyurethane membrane. Each seeding density of KC and FB were equal. Several days after the co-cultivation, the mixed culture sheet was transferred to a freshly harvested STSG donor wound in an extensive burn patient. A polyurethane membrane without cells was used for control coverage. Seven patients were treated using the mixed culture sheets. Autologous cells were used in 3 cases, and allogenic cells were used in the other 4 cases. The time for the final epithelization was determined. In vitro production of several growth factors by a cellular sheet was also investigated by ELASA.

Results and Conclusion: TGF- α , EGF, TGF- β , VEGF were well produced by the mixed culture sheet. The time for final epithelization of the wounds was reduced by 2.5 days with the mixed culture sheet application as compared to the control sites. It was suggested that the mixed culture sheet may be useful for the treatment of STSG donor wounds.

025

CLINICAL APPLICATION OF AUTOLOGOUS CULTURED DERMAL SUBSTITUTES FOR GIANT CONGENITAL PIGMENTED NEVUS

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This study was designed to try the application of autologous cultured dermal substitute (CDS) in conjunction with patient's own epidermis. The autologous CDS was prepared by seeding cultured autologous fibroblasts on the spongy matrix of hyaluronic acid and atelo-collagen. A 9-year-old man with a giant congenital pigmented nevus (intradermal type) was included in this clinical study. A part of nevus (30 cm \times 10 cm) was excised superficially at a thickness of 20/1000 inches, and followed by second excision to remove nevus at a level of full-thickness skin. The autologous CDS was applied to the debrided wound surface. The split-thickness skin obtained by first excision was preserved at 4°C. After 1 week, the epidermis was obtained from the preserved split-thickness skin using dispase, and followed by grafting on the wound bed, which was prepared by applying autologous CDS. This patient's own epidermis was found to take permanently, achieving an excellent clinical results.

026

TREATMENT WITH AUTOLOGOUS CULTURED DERMAL SUBSTITUTES (CDS) FOR BURN SCAR CONTRACTURE IN CHILDREN

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Aim: A treatment of extensive burn contracture in children needs to repeat the autologous skin graft. This study was designed to evaluate the application of autologous CDS to prepare the proper wound bed acceptable for the split-thickness autologous skin graft.

Methods: Prior to the clinical study, the master cell banking system was established using a small piece of skin derived from each patient. The autologous CDS was prepared by plating the patient's own fibroblasts, cultured from the master cells, on a spongy matrix of hyaluronic acid and atelo-collagen. The CDS was applied on the skin defects left behind surgical excision to release scar contracture. The split-thickness autologous skin graft (6~8/1000 inch) was applied on the wound bed, prepared by using the CDS.

Results: The clinical trials were conducted in 5 cases. When the autologous CDS was applied on the skin defect, exposing subcutaneous fatty tissue, the highly vascularized wound bed was prepared within about 1 week. Although a split-thickness skin graft was very thin, the severe contracture was not observed over a period of several months.

Conclusion: The application of autologous CDS is promising for the treatment for extensive burn scar contracture in children.

Allograft Cultured Skin

Moderated by Yoshimitsu Kuroyanagi

028

CLINICAL TRIALS OF ALLOGENIC CULTURED DERMAL SUBSTITUTE FOR THE TREATMENT OF BURN WOUNDS

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Aim: Recently, various types of cultured skin substitutes have been developed and some of them are used clinically. This study was designed to evaluate the efficacy of allogenic cultured dermal substitute which was applied to burn injuries in clinical trials such as deep dermal burns and dermal burns.

Methods: Allogenic cultured dermal substitute (CDS) was simply applied to the burn wound, over which covering materials were applied to protect CDS.

Results: The application of CDS to deep dermal burns was proved to facilitate healthy granulation tissue formation at early stage and epithelialization from the outer margins. When CDS applied to the debrided wound surface of dermal burns, an excellent wound bed was generated which was suitable for the graft take of an autologous patch.

Conclusion: CDS provides an excellent epithelialization and granulation for burn wounds.

029

TREATMENT OF FRESH DDB WOUNDS WITH ALLOGENIC CULTURED FIBROBLASTS, ALLOGENIC CULTURED DERMAL SUBSTITUTE

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Aim: Multi-center's clinical study for the application of the cultured dermal substitute (CDS) composed of hyaluronic acid and collagen spongy matrix with allogenic cultured fibroblasts¹ is in progress since 2001. In this study, effect of the CDS for the treatment of fresh DDB wounds was tested.

Methods: Six cases of second-degree burns, diagnosed as DDB by observation of blood stream of the dermal capillary using Compact Micro Vision System (Hi-Scope[®], Hi Rox Co., Japan), were treated with the cultured allogenic fibroblasts, CDS.

Results: The epithelialization of the wounds was obtained on 8.2 ± 2.6 (mean \pm SD) days after the application.

Conclusion: The results of our clinical experiences suggest that the allogenic cultured fibroblasts have beneficial effect on the wound healing of the DDB.

References: 1)Kuroyanagi Y, Yamada N, Yamasita R *et al*; Tissue-engineered product: allogenic cultured dermal substitute composed of spongy collagen with fibroblasts. *Artif Organs* 25(3): 180-186, 2001

030

CLINICAL TRIALS WITH ALLOGENEIC CULTURED DERMAL SUBSTITUTES (CDS) (REGENERATING MEDICAL MILLENNIUM PROJECT OF THE MINISTRY OF HEALTH, LABOR AND WELFARE)

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Aim: Clinical researches using allogeneic CDS, developed in the R & D Center for Artificial Skin of Kitasato University, have been carried out in 30 medical centers across Japan. The clinical results in our hospital, especially focusing on the treatment of refractory ulcers and dermal burns, were reported in this study.

Methods: The CDS was prepared by plating cultured fibroblasts on a spongy matrix of hyaluronic acid and atelo-collagen. The CDS was used in 13 clinical trials, including 6 refractory ulcers, 4 dermal burns, 1 skin defect left behind preparing skin flaps, and 2 skin defects left behind removal of scar. The use of CDS in conjunction with a conventional ointment-gauze dressing was repeated at an interval of 4 to 7 days over a period of 2 to 6 weeks.

Results: The successful application with CDS was achieved in all cases. Especially, in case of refractory ulcers, failed to heal even by using trafermin (Fiblast spray[®]) or other ointments, a complete healing was achieved in one case and wound size reduction was observed in other 5 cases within 6 weeks.

Conclusions: Our results suggest that the CDS is effective in the treatment of refractory ulcers and other skin defects. The excellent clinical results seem to be related to the cytokines released from the CDS.

031

CLINICAL TRIALS WITH ALLOGENEIC CULTURED DERMAL SUBSTITUTES FOR THE TREATMENT OF BURNS AND SKIN ULCERS (REGENERATING MEDICAL MILLENNIUM PROJECT OF THE MINISTRY OF HEALTH, LABOR AND WELFARE)

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Aim: Multi-center clinical trials were performed using allogeneic cultured dermal substitutes (CDS) newly developed in the R & D Center for Artificial Skin of Kitasato University.

Method: This study was designed to evaluate the efficacy of CDS for the treatment of burns in 5 cases and the treatment of skin ulcers in 4 cases. Complications with the skin ulcers were diabetes (1 case), varix (1 case), and collagen diseases (2 cases). The wound surface area was measured with picture analysis software during the wound healing process.

Results: The clinical evaluation was made using a protocol that includes a standard for the development of new wound dressings. According to the comprehensive judgment, the results achieved were evaluated as excellent for 4 burn cases and good for one case. Regarding the skin ulcers, results were excellent in 3 cases and good for one case. No harmful complications were observed during this study.

Conclusions: CDS were useful for burn and ulcer treatment but the wound surface must be checked rigorously for the occurrence of infection during the healing process.

References: 1.Kuroyanagi Y, Yamada N, Yamashita R. *et al*; Tissue-engineered product: Allogenic cultured dermal substitute composed of spongy collagen with fibroblasts. *Artificial Organs* 25:180-186, 2001

032

TREATMENT OF CHRONIC ULCER WITH ALLOGENEIC CULTURED DERMAL SUBSTITUTE (CDS) COMPOSED OF COLLAGEN SPONGE WITH FIBROBLASTS

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We have been cultivating human epidermal cells for therapeutic purpose according to the original methods developed by Rheinwald and Green. Cultured epithelium (CE) was applied to patients with severe skin defects, burn wounds, chronic skin ulcers and cutaneous disorders like hypomelanosis. Autologous CE allows to restore massive skin surface in a short period compared with other conventional treatments. For grafts take, it is important to manage wound beds properly prior to CE grafting. The CDS was applied to prepare wound bed acceptable for CE grafting. The CDS was designed to secrete various types of cytokines, i.e., VEGF and KGF to stimulate wound healing. The successful management of deep wounds like chronic skin ulcer or burn ulcer requires granulation tissue formation and epithelialization to wound closure. This study aimed to evaluate the application of CDS in conjunction with CE for patients with chronic skin ulcer and burn ulcer. In some cases the wounds were cured by using CDS, and followed by CE grafting. All clinical trials achieved excellent or good results, showing no contracture and no hypertrophic scar after wound closure. The CDS was found to be useful to prepare wound beds and to facilitate wound management.

033

CLINICAL TRIALS WITH ALLOGENEIC CULTURED DERMAL SUBSTITUTES (CDS) FOR TREATMENT OF SKIN ULCERS (REGENERATING MEDICAL MILLENNIUM PROJECT OF THE MINISTRY OF HEALTH, LABOR AND WELFARE)

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Aim: Multi-center's clinical study, using allogeneic CDS are proceeded in 30 hospitals as the Millennium Project of the Ministry of Health, Labor and Welfare. The clinical trial in our hospital was designed to evaluate the efficacy of CDS for the treatment of refractory skin ulcers.

Methods: The application of CDS on the debrided wound was repeated at an interval of 3 to 5 days. The evaluation was performed according to the protocol, especially focused on granulation tissue formation, wound size reduction, and epithelialization.

Results: The clinical trials were conducted in 6 cases, including 5 cases with various complications, i.e., diabetes (2 cases), collagen diseases (1 case), renal failure (1 case), and skin defect after resection of tumor (1 case). According to the comprehensive judgement, 5 cases were evaluated as achieving excellent, and 1 case was evaluated as achieving good.

Conclusion: The successful application of CDS is dependent on the control of wound conditions free from infection. Appropriate debridement was necessary prior to application of CDS.

034

APPLICATION OF ALLOGENEIC CULTURED DERMAL SUBSTITUTE TO SKIN DEFECTS AFTER EXCISION OF SKIN CANCER

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Aim: This study was aimed to confirm the usefulness of allogeneic cultured dermal substitute (CDS) in the treatment of skin defects after excision of skin cancer.

Methods: Eleven elderly patients (mean age of 76.3 year old) with skin cancer were included in this study. Most of the patients had basic disease such as diabetes or ASO. Allogeneic CDS used in the study were produced at Kitasato University. The CDS were applied to skin defects with exposing bone or tendon after oncological surgery, and changed once or twice a week until the open wound became suitable for autologous skin grafting or healed completely.

Results: Healthy granulation tissue was formed to cover the exposed bone or tendon in all cases. Only in one case, the treatment with allogeneic CDS was abandoned owing to undesirable infection. In eight cases, appropriate wound beds acceptable for autologous skin graft were prepared. In other two cases, the skin defect became smaller and eventually closed without skin graft. In ten cases with or without skin graft, undesirable scar contracture was not observed over prolonged follow-up.

Conclusions: Elderly patients with skin cancer provide reconstructive surgeons with challenging problems when bone or bare tendon is exposed after oncological surgery. Flap transfer might be complicated especially when the patients suffered from a basic disease such as diabetes or ASO. This study has confirmed that the use of allogeneic CDS is a safe and reliable method to achieve wound healing in those high-risk patients.

Laser

Moderated by Eiju Uchinuma

035

FLAT RESECTION TECHNIQUE BY CO₂ LASER FOR LENTIGO

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It has been said that CO₂ laser vaporization and resection conventionally performed for elevated lentigo may cause depressed scar formation when the diameter of the lesion exceeds 5 mm. Accordingly, in consideration of cosmetic factors, we have devised a method for the removal of even a larger elevated lentigo by the Flat Resection technique which can be used effectively without causing a depressed scar to form. Upon the primary laser irradiation, the lesion was resected at the horizontal plane of the skin, and the resected tumor was subjected to histopathological analysis. If the lesion remained about one month after the primary irradiation, additional laser treatment was performed at the patient's request. From our experience, satisfactory outcomes were obtained for up to 12-mm lesions. Following the flat resection, residual lesions were pathologically observed in 52.5%, and macroscopically observed in 45%. Additional laser treatment was performed for 35%. It was considered that this technique is useful for sites such as the base of ala nasi and the vicinity of the eyebrow, of which simple reefing requires skills. However, since the possibility of malignant degeneration of lentigo could not be ruled out in a longer-term follow-up, particular care is needed to select a case suitable for this technique.

036

A NEW METHOD FOR SKIN ABRASION THERAPY USING THE PULSED CARBON DIOXIDE LASER WITH SCANNERYuki Iijima,* Tatsunori Tanaka,* Kei Yoneda,** Eiji Miyamoto*,**
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We have treated superficial skin lesions using pulsed carbon dioxide laser with scanner (NIDEK COL-1040). We can choose the shape, size and power of irradiation programmed in the scanner. It makes the treatment more effective and uniform to combine the scanner mode with the pulse mode. However, for a lesion of complicated shape, it is difficult to avoid skin damage caused by multiple irradiations to a small area and/or irradiation to the normal skin. The result may be unsatisfactory with pigmentation or unsightly scar in such case. As the water-solvability is a characteristic of the carbon dioxide laser beam, we used water film around the lesion to avoid unexpected irradiation to the normal skin. This method provided us better results.

037

EXPERIENCE OF USE OF LONG PULSE ALEXANDRITE LASER FOR FRESH FACIAL TRAUMATIC TATTOONaohiro Ishii, MD,¹ Masashi Fukatsu, MD, Ph.D.,¹ Yuji Nakanishi, MD, Ph.D.²
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Objectives: We devised a method of irradiating a fresh traumatic tattoo with long pulse alexandrite laser in conjunction with the use of a dynamic cooling device (DCD) to minimize the injury of the epidermal layer before the occurrence of pigmentation in the deep layer of the skin, when the wound is not completely epithelialized.

Methods: A 4-year-old girl suffered a burn injury and deposition of fuel residues in a diameter of 5 cm on the left cheek by backfire of a motorcycle muffler. The patient was treated with an ointment at a hospital for about 10 days, and then referred to our hospital. On initial visit, removal of foreign bodies was attempted by brushing but with little success. Accordingly, long pulse alexandrite laser treatment was performed four times in total at intervals of about one week. On the initial irradiation DCD was not used, but was used on all subsequent treatments thereafter for the protection of the epidermal layer.

Results: Since remarkable pigment reduction was observed after the laser irradiation, the treatment was stopped. Follow-up was performed while the lesion was shielded from light.

Conclusion: Since the long pulse alexandrite laser with the DCD conveniently enables continuous irradiation of pigments in a short time while minimizing damage to the epidermis, its therapeutic effect can be expected. However, great care is needed during the treatment to prevent scarring.

Angioneogenesis • Blood Flow*Moderated by Kazuki Ueda*

038

NEOVASCULARIZATION IN ARTIFICIAL DERMIS AFTER SECONDARY SKIN GRAFTING-SCANNING ELECTRON MICROSCOPIC OBSERVATION OF MOLDED BLOOD VESSELS-Junko Yasuda, Shigehiko Kawakami
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Aim: This study observed neovascularization in an artificial dermis after secondary skin grafting both light microscopically and electron microscopically and examined the optimal time of operation for secondary skin grafting through change in form of the graft.

Method: Using 31 adult rabbits, full-thickness skin defects were produced inside the ear. Artificial dermis was fixed on one side of the wounds (AD group). On the other side of the wound, artificial dermis was not used (Control group). On the fifth, seventh, and tenth day, thin split-thickness skin was taken from the back of a rabbit, and grafted.

Result: It mainly observed about the tenth day because more than 60% of grafts exhibited complete take on the tenth day, while on the fifth or seventh day no taking was observed. Electron microscopic findings revealed that, in the AD group before transplant, neovascularization was seen, extending from the bed to artificial dermis vertically, but there was no angiogenesis observed extending from the wound margin centripetally. This centripetal angiogenesis was observed in the control group and seemed to indicated the contraction of the wound and grafted skin.

Conclusion: It was concluded that the most suitable period for secondary skin grafting in the experiment model was 10 days after implantation of artificial dermis.

039

OBSERVATION OF ANGIOGENESIS IN GRANULATION TISSUE USING THE DRESSING MATERIALKazuaki Murashita, M.D.,^a Hiroko Sinzawa, M.D.,^a Akira Takeda, M.D.^b and Eiju Uchinuma, M.D.^c^a Department of Plastic and Reconstructive Surgery, Chigasaki Tokushukai Medical Center^b Department of Plastic and Reconstructive Surgery, Yokohama Kowan City Hospital^c Department of Plastic and Reconstructive Surgery, Kitasato University School of Medicine

Aim: It is important to analyze the angiogenesis and the formation of the granulation tissue in understanding the wound healing process. The dressing material is thought that the wound healing is promoted, and understanding the effect of the dressing material. The model which used the dressing material was made, and the angiogenesis in the granulation tissue formation process were compared.

Method: The Sprague-Dawley rat's granulation formation model and the model which used the dressing materials were made. The granulation tissue was removed on the 7th day, the 14th day, the 28th day, and the 42nd day. The angiogenesis and the formation of network of newly formed vessels during the granulation tissue formation were observed with Confocal laser scanning microscopy.

Result: The angiogenesis starts from the depths part of the granulation tissue. Afterwards, it progresses superficial part of the granulation tissue, and the network of the blood vessel is formed. In this process, as the model which used the dressing material, the network of the angiogenesis was admitted from early time.

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040

THE FUNCTION OF 'CHOKE VESSELS' TO THE BLOOD FLOW: ANGIOGRAPHIC AND LASER FLOW-GRAPHIC STUDY ON THE RAT FLAP MODEL

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Aim: The 'choke vessels', defined by Taylor [1], are reduced-caliber anastomosing vessels which are normally the barrier in the vasculature. They also have capacity to dilate and increase the blood flow. The aim of this study is to investigate the anatomical and dynamic function of choke vessels.

Study design: The 3-territory bipedicle flaps were elevated on the back of the Wistar rats [2]. The flaps were directly sutured back in place in the revascularized group (10 animals), and the plastic sheets were underlaid in the non-revascularized group (10 animals). On the day 3, each flap was converted to the mono-pedicle flap and was scanned by the laser flow-graphy and injected for the angiography.

Results: All flaps survived completely. The revascularized flaps showed evenly dilated choke vessels in the 2 choke zones of the flaps and the blood flow from the vascular pedicle covered more than 2 territories. The non-revascularized flaps showed more remarkable but uneven dilatation of choke vessels between 2 choke zones. The blood flow in non-revascularized flaps, however, reached only 1 territory, blocked by the first choke zone which dilated less than the other zone.

Conclusions: By dilating, the choke vessels are activated and can have the adverse function of regulating the blood flow, namely, suppression and escalation of the blood flow.

References: 1. Taylor GI, Palmer JH: The vascular territories (angiosomes) of the body: Experimental study and clinical applications. *Br. J. Plast. Surg.* 40: 113, 1987.
2. Taylor GI, Minabe T: The angiosomes of the mammals and other vertebrates. *Plast. Reconstr. Surg.* 89: 181, 1992.

041

NEW EXPERIMENTAL ANIMAL MODEL OF PRESSURE ULCERS USING THE ORIGINALLY DESIGNED SKINFOLD CHAMBER

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It is clear that the interruption of microcirculation is one of the significant factors to the pathophysiology of pressure ulcers. But the relation between them has not sufficiently elucidated yet. We aimed at developing the experimental animal model of pressure ulcers. Using the originally designed skinfold chamber and pressing systems, we visualized interruption, remodeling and angiogenesis of microcirculation dynamically. And such various microcirculatory responses also could be evaluated and recorded chronically. We compared ischemia-induced injury to ischemia-reperfusion-induced injury with this model, and analyzed each reaction of microcirculation quantitatively by measuring the functional capillary density. This model can be useful from the viewpoint of microcirculation.

042

COMPARATIVE EVALUATION OF DUPLEX-DERIVED PARAMETERS IN PATIENTS WITH CHRONIC VENOUS INSUFFICIENCY: CORRELATION WITH CLINICAL MANIFESTATION

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Aim: To compare the duplex-derived parameters between patients with early, and those with advanced chronic venous insufficiency (CVI), and to determine the indicative parameters reflecting the progression of CVI.

Methods: A total of 1132 limbs in 914 patients with of primary valvular incompetence were included. The clinical manifestations were categorized according to the CEAP (clinical, etiologic, anatomic, and pathophysiologic) classification, and the patients were divided into two groups: group I ($C_{1-2}E_{p,A_{3-4}D_{p,P}_{1-2}}$) and group II ($C_{1-4}E_{p,A_{3-4}D_{p,P}_{1-2}}$). The distribution of venous insufficiency was determined, and the parameters assessed were the duration of reflux (s), the peak reflux velocity (cm/s), and the flow at peak reflux (ml/s).

Results: There was no significant difference in overall superficial venous reflux between the groups. And the frequency of isolated deep and perforator incompetence did not differ between the groups. The duration of reflux did not improve the discrimination power between the groups. In contrast, the peak reflux velocity had significant discrimination power at the sapheno-femoral junction (SFJ, $p < 0.0001$) and sapheno-popliteal junction (SPJ, $p = 0.0002$), and in the greater saphenous vein (GSV, $p < 0.0001$), in the superficial femoral vein (SFV, $p = 0.0041$), and popliteal vein (POPV, $p = 0.003$). The peak reflux flow was significantly higher in group II at the SFJ ($p < 0.0001$) and SPJ ($p = 0.0029$), and in the GSV ($p < 0.0001$), in the common femoral vein ($p = 0.006$), in the SFV ($p = 0.0005$), and POPV ($p = 0.0003$).

Conclusions: Superficial venous insufficiency may play a major role in the disease development of advanced CVI. The peak reflux velocity and peak reflux volume improve discrimination power between early-stage and advanced CVI.

References: 1. Yamaki T, Nozaki M, Fujiwara O, Yoshida E. Comparative evaluation of duplex-derived parameters in patients with chronic venous insufficiency: correlation with clinical manifestations. *J Am Coll Surg* 2002; 195(6): 822-830

043

EFFECTS OF EARLY WOUND EXCISION ON HEPATIC BLOOD IN THE EXTENSIVELY BURNED RAT

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Aim: Effects of early excision on hepatic blood flow were investigated in burned rats.

Methods: 30% TBSA burned Wistar rats were allocated to four groups, infusion group (n = 13): fluid resuscitation with lactated Ringer's; early excision group (n = 13): early escherectomy and allogenic skin grafting with fluid resuscitation; burn group (n = 13): burn injury alone without any treatment; sham group (n = 15): sham burn procedure. The hepatic blood flow was measured at 0, 0.5, 1, 2, 3, 6, 12 and 24 hours postburn. At 12 and 24 hours postburn, plasma Endothelin-1 (ET-1) and nitric oxide (NO) levels were detected.

Results: In the infusion and the burn group, a significant decrease in hepatic blood flow compared with the sham group. In contrast in the early excision group, the hepatic blood flow was not decreased and there was a significant difference from the infusion group at 12 hours postburn. The plasma ET-1 and NO levels were markedly elevated in the infusion and the burn group at 12 and 24 hours postburn. Whereas in the early excision group, no significant increase either in the plasma ET-1 or NO levels were found compared with the sham group during experimental period.

Conclusion: The hepatic blood flow decreases after a massive burn injury even with adequate fluid resuscitation. Early excision is helpful in stabilizing the hepatic blood flow without further aggravating the hepatic hemodynamic load. The decrease in plasma ET-1 and NO levels after early wound excision procedure may be playing a role in improving the hepatic blood flow.

Miscellaneous

Moderated by Yasunori Okada

044

THE DIFFERENCE IN VIRULENCE BETWEEN CLINICALLY RECOVERED STAPHYLOCOCCUS AUREUS AND AN ESTABLISHED STRAIN APPLIED TO A SKIN ULCER MODELMasahiro Tachi, Shinichi Hirabayashi
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Aim: We attempted to establish a rat model by placing gauze on a wound bed. We then used this model to evaluate the virulence of an established strain of *Staphylococcus Aureus* and a clinical strain isolated from a patient with a wound infection.

Methods: Using a 1.5×1.5 cm template, full thickness skin wounds were made through the panniculus carnosus on the backs of rats. A small piece of gauze was placed over each wound. The wound beds were inoculated with 1.5×10^6 cfu of *Staphylococcus Aureus* from the American Type Culture Collection, or 1.5×10^9 cfu of a clinical isolate. The wounds were debrided every 48 hours and samples taken to the laboratory for quantitative bacteriologic analysis. Specimens were fixed and stained by H/E and Gram staining.

Results: The clinical strain of *Staphylococcus Aureus* resulted in bacterial counts (cfu per gram of tissue) exceeding 10^7 on days 2 to 6, while the established strain produced bacterial counts exceeding 10^7 cfu on days 2 to 4 only. Gram stain analysis revealed that the clinical strain penetrated deep granulation tissue, whereas, the established strain was present in small cell nests at the wound margins.

Conclusion: A clinical strain isolated from a patient showed deeper penetration of the wound bed than an established strain, resulting in prolonged elevation of the bacterial count.

045

ALTERATION OF INTEGRIN EXPRESSION IN MIGRATING EPIDERMAL CELLS AND FIBRONECTIN DISTRIBUTION IN THE DERMIS WITH PRESSURE ULCERSMiyoko Kubo, Mika Shinoyama, Kousaku Suenobu, Takahiko Moriguchi
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Failure of re-epithelialization characterizes the pathophysiology of chronic wounds. However, the mechanism of this failure has not been fully clarified. During the re-epithelialization of acute wounds, migrating epidermal cells up-regulate integrins $\alpha 5\beta 1$, $\alpha v\beta 5$, $\alpha v\beta 6$ and these integrins play an important role in the re-epithelialization process. Laminin-1 is a basement membrane component which appears during the late phase of the re-epithelialization process. In this study, with 6 specimens from burn patients (acute wounds, control) and 11 specimens from patients with pressure ulcers, we examined the expression of $\alpha 5\beta 1$ in the migrating epidermis, laminin-1 at the dermal-epidermal junction and fibronectin in the dermis by an immunohistochemical method. We then compared the findings for $\alpha 5\beta 1$ with histological, laminin-1 and fibronectin distribution findings. In 8 out of the 11 pressure ulcer specimens, the expression of $\alpha 5\beta 1$ significantly decreased or was negative while it increased in the control. The degree of expression correlated well with histological findings of epidermal elongation over the wound bed. Moreover, fibronectin distribution in all the pressure ulcer specimens decreased considerably while it increased in three out of the six burn specimens. A statistically significant correlation was found between the distance at which the cells were positively stained for $\alpha 5\beta 1$ and the distance at which they were negatively stained for laminin-1 in both the burn (control) and pressure ulcer specimens. Our data demonstrated that the decrease in $\alpha 5\beta 1$ expression correlated with the failure in re-epithelialization of chronic wounds (pressure ulcers). It was also suggested that the decrease in fibronectin distribution was responsible for the failure of the re-epithelialization process.

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ALLOGENEIC TRANSPLANTATION OF GENETICALLY MODIFIED PRIMATE EMBRYONIC STEM CELLSAsano T,^{1,2} Hanazono Y,² Sasaki K,^{1,2} Ueda Y,³ Hasegawa M,³ Ageyama N,⁴ Terao K,⁴ Kitano Y,⁵ Momoeda M,⁶ Ozawa K,⁷ Harii K,¹
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Aim: To examine the efficacy and safety of human embryonic stem (ES) cell-based therapies, allogeneic transplantation of monkey ES cells would be useful. We transplanted genetically marked monkey ES cells into the allogeneic fetus.

Methods and Results: Cynomolgus ES cells were transduced once using a simian immunodeficiency virus-based lentivirus vector encoding the GFP gene driven by the CMV promoter at 1, 10 and 100 transducing units per cell. Five days posttransduction, 60, 80 and 90% of the cells expressed GFP, respectively, and the expression levels were stable for 5 months. GFP expression was still observed after embryoid-body formation. The gene-marked ES cells were transplanted into the cynomolgus fetus in the abdominal cavity (n=2) or liver (n=1) after the first trimester. The fetuses were delivered 1 month posttransplantation. Transplanted cell progeny were detected (~1%) in multiple tissues by quantitative PCR and in situ PCR of the GFP sequence. No teratoma was found in the tissues. **Conclusions:** Cynomolgus ES cells can be engrafted in the allogeneic fetus. We are now trying to transplant cynomolgus ES cells differentiated to neural or hematopoietic lineage.