



Abstracts

The 37th Annual Meeting of the Japanese Society for Wound Healing

Yokohama Royal Park Hotel, Yokohama, Japan
December 6–7, 2007

Congress President: Hiroshi Shimada, M, Ph Professor, Department of Gastroenterological Surgery, Yokohama City University

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

GENERAL SESSION 1: WOUND HEALING

05

Impacts of peripheral blood and bone marrow aspirate derived platelet rich plasma on the wound healing in chronic ischemic limb

Nishimoto S, Kawai K, Tsumano T, Fukuda K, Kageyama S, Yokoyama S, Fujita K, Oguma T, Kakibuchi M
Department of Plastic Surgery, Hyogo College of Medicine

Aim: Platelet rich plasma (PRP) has attracted attention as a safe and cost-effective source of growth factors that stimulate cells to regenerate tissue. With the same technique to concentrate PRP from peripheral blood, bone marrow aspirate was processed and marrow cells were concentrated as well as platelets. (*Wound Repair Regen.* 2007;15(1)) Impacts of PRP's derived from peripheral blood (pb-PRP) and from bone marrow aspirate (bm-PRP) on wound healing of rabbits' ischemic limbs were studied.

Methods: A superficial femoral artery was ligated and excised from a rabbit's hind-limb. Three weeks after, when the inflammation of primary surgery was supposed to have faded away, 2×2 cm of full-thickness skin defects were made on the ischemic and undisturbed limb. 200 micro-liter of saline was injected into the wound floor of non-ischemic limb. In ischemic-saline group, 200 micro-liter of saline was injected into the wound on ischemic limb. In pb-PRP group, 200 micro-liter of PRP processed from autologous peripheral blood was injected the wound on ischemic limb. In bm-PRP group, 200 micro-liter of bm-PRP, derived from autologous iliac bone marrow aspirate was injected. The skin defects were observed with the time course and recorded with a digital camera. Area of skin defect was measured with an image analysis software.

Results: Skin defected areas on the ischemic limbs were significantly wider than those on non-ischemic limbs on day 7 and 21. pb-PRP group could not show any significance with ischemic-saline group. bm-PRP group showed significantly small skin defect area comparing with pb-PRP and ischemic-saline group at all time points.

Conclusion: Wound healing on an ischemic limb was accelerated with bm-PRP, whereas pb-PRP could not show any significance from saline.

06

Polarized light emitting diode (LED) irradiation increases fibroblast proliferation and accelerates wound healing

Tada K, Hashimoto N, Ikeda K, Tomita K
Department of Orthopaedic Surgery, School of Medicine, Kanazawa University, Kanazawa, Japan

Aim: We examined the effect of polarized LED irradiation on fibroblast proliferation and wound healing with a high intensity LED irradiation device set at 627 nm.

Methods: Five groups were classified as control (C), non-polarized (N), linear (L), right-hand circular (RC), and left-hand circular (LC). *In vitro* study, fibroblast cell cultures were irradiated with a range of 1 to 10 J/cm², and cellular proliferation was evaluated with a WST-8 assay. *In vivo* study, two round, full-thickness wounds 2 cm in diameter were produced on the dorsal side of rats. On day 7 and 14, the ratio of the residual wound area expressed as a percentage to the control group was measured and expression of type I procollagen mRNA in scar tissue was determined by RT-PCR.

Results: Irradiation with 4 J/cm² increased cellular proliferation significantly when measured 48 hours after subculture. In particular, the proliferation rates of RC and L groups were 116% and 113%, respectively. In addition, wound healing was accelerated significantly. On day 7, the ratio of the residual wound areas of RC and L groups were 72% and 69%, respectively. Expression of type I procollagen mRNA in the RC group was significantly increased about 1.5-fold in comparison to the control group.

Conclusions: These experiments demonstrate that right-hand circular and linear polarized light from LED is more effective than non-polarized light.

10

The effect of 1% PVP-I solution for experimentally heavily colonized wounds

Emi Kanno¹, Masahiro Tachi², Lianbo Zhang², Sohachi Toriyabe², Masahiro Kosaka²

¹Tohoku University School of Health Sciences

²Department of Plastic and Reconstructive Surgery, Graduate School of Medicine, Tohoku University

Chronic skin wounds are usually colonized with bacteria, then subsequent infection may develop. Topical antiseptics are commonly used to control bacterial colonization. The use of 1% polyvinylpyrrolidone-iodine (PVP-I), a topical antiseptic, on chronic open skin wounds remains controversial in clinical setting because of its cytotoxicity. Here we tested 1% PVP-I solution, saline, to determine if it reduce bacterial count on the wound surface and within the tissue that may lead to wound reduction. Open wounds were created on the backs of SD rats, suspensions of *P. aeruginosa* were applied on wounds. Wounds were kept in closed environment during this experiment. After 2 days postwounding, wounds were irrigated using 10 ml syringe and spray tip every day. Our results indicate that 1% PVP-I irrigation resulted in reduced bacterial count on the wound surface and within the tissue compare to saline irrigation. 1% PVP-I irrigation promoted wound reduction compared with saline irrigation, but it did not reach significance. In conclusion, irrigation using 1% PVP-I reduced bacterial count on the wound surface, and allowed the wound to progress to healing.

GENERAL SESSION 2: BASIC RESEARCH

13

Deliberation of the concentration of basic fibroblast growth factor impregnated into collagen/gelatin sponges

Norikazu Kanda, Satoru Takemoto, Morimoto Naoki, Shigehiko Suzuki
Department of Plastic and Reconstructive Surgery, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

Aim: We have developed gelatin/collagen sponges impregnated with basic fibroblast growth factor (bFGF) for sustained-release and reported that the collagen/gelatin sponge with 10wt% gelatin (GCS) was most effective. The objective of this study was to deliberate optimal concentration of bFGF impregnated into GCS for acceleration of wound healing.

Methods: GCSs impregnated with saline, bFGF (1 µg/cm², 7 µg/cm², and 14 µg/cm²) were implanted into full-thickness skin defects (diameter 8mm) on the

backs of mice (C57BL). The wound area and the length of neoepithelium were evaluated at 1, 2, and 3 weeks after implantation.

Results: The wound areas were significantly decreased in the groups treated with GCSs impregnated with bFGF7, $14 \mu\text{g}/\text{cm}^2$. The lengths of neoepithelium were significantly longer in the group treated with GCSs impregnated with bFGF7 $\mu\text{g}/\text{cm}^2$.

Conclusions: In this study, GCSs impregnated with bFGF7 $\mu\text{g}/\text{cm}^2$ promoted wound healing.

References:

1. Kawai K, et al. Accelerated tissue regeneration through incorporation of basic fibroblast growth factor-impregnated gelatin microspheres into artificial dermis. *Biomaterials*. 2000; 21: 489–499
2. Kawai K, et al. Accelerated wound healing through the incorporation of basic fibroblast growth factor-impregnated gelatin microspheres into artificial dermis using a pressure-induced decubitus ulcer model in genetically diabetic mice. *British Journal of Plastic Surgery* 2005; 58: 1115–1123
3. Tsuji-Saso Y, et al. Incorporation of basic fibroblast growth factor into pre-confluent cultured skin substitute to accelerate neovascularisation and skin reconstruction after transplantation. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery* 2007; 41: 228–235
4. Tanaka E, et al. Mechanism of acceleration of wound healing by basic fibroblast growth factor in genetically diabetic mice. *Biol. Pharm. Bull.* 2005; 19: 1441–1448
5. Okumura M, et al. Acceleration of wound healing in diabetic mice by basic fibroblast growth factor. *Biol. Pharm. Bull* 1996; 19: 530–535

14

Effect of epidermal growth factor on enhancing fibroblast proliferation

Shintaro Yamada, Mami Yamazaki, Yoshimitsu Kuroyanagi
R & D Center for Artificial Skin, Kitasato University, Sagami-hara, Kanagawa, Japan

Aim: This study was designed to investigate the optimal concentration of epidermal growth factor (EGF) to enhance fibroblast proliferation in culture medium containing a given amount of EGF in a flask. In addition, the effect of EGF on proliferation of fibroblasts incorporated in a collagen gel was studied. **Methods:** Fibroblasts were seeded in a flask at a density of 1×10^4 cells/cm² and then the behavior of cell proliferation was observed in culture medium (DMEM supplemented with 10% FBS) containing EGF ranged from 2 to 50 μg . Fibroblasts were incorporated in a collagen gel at a density of 2×10^5 cells/cm² and then the behavior of cell proliferation was also observed in culture medium containing EGF.

Results: The number of fibroblasts in a culture medium containing EGF resulted in about 3-fold number of control group in culture medium without EGF. The fibroblasts in culture medium containing EGF resulted in a morphological change to a compact, smaller shape. The number of fibroblasts incorporated in a collagen gel was found to increase slightly in cultured medium containing EGF.

Conclusion: EGF has a potency to enhance the cell proliferation of epithelial cells and other types of cells. EGF is considered to be promising as a component of wound dressing, since EGF appears to function similarly to bFGF for wound healing. The addition of EGF into culture medium is useful in preparing a cultured dermal substitute composed of fibroblasts and collagen gel.

16

Effects of cyclosporin A on extracellular matrix metabolism by human dermal fibroblasts

Abe M, Yokoyama Y, Syuto T, Ishibuchi H, Ishikawa O
Department of Dermatology, Gunma University Graduate School of Medicine, Maebashi, Japan

Although the actions of cyclosporin (CyA) on the keratinocyte are well established, little is known about its effects on dermal fibroblasts. IL-6 is one of inflammatory cytokine playing a pivotal role in certain skin diseases such as dermal wound healing as well as psoriasis. The aim of this study was to determine whether or not CyA modifies the metabolism of extracellular matrix (ECM) by human fibroblasts *in vitro*. CyA altered the morphology of fibroblasts in the collagen matrix. Fibroblast proliferation was suppressed by CyA at 100 and 100 ng/ml. The production of type I collagen and TIMP-1 was also suppressed by CyA at 1000 ng/ml, and costimulation with IL-6 enhanced the decreased production at 1000 and 100 ng/ml of CyA. The production of MMP-1 was also suppressed by CyA in a dose-dependent manner. In contrast, the decreased production of MMP-1 was restored at CyA 0.1~100 ng/ml in the pres-

ence of IL-6. Regardless of the presence or absence of IL-6, the production of MMP-2 decreased at 1000 and 100 ng/ml, whereas the production of MMP-9 was unchanged. The production of TGF- β decreased at 100 ng/ml. This study indicates that CyA may influence the ECM metabolism and proliferation of human dermal fibroblasts, and effects of CyA were modulated by IL-6. This preliminary study suggests the possibility that CyA, in part, improves dermal wound healing by regulating the remodeling of ECM as well as by the actions on immunocompetent cells.

33

The relationship between hemidesmosomes and focal contacts in motile keratinocytes

Toshiyuki Ozawa,¹ Daisuke Tsuruta,² Hiromi Kobayashi,² Teruichi Harada,¹ and Masamitsu Ishii^{1,2}

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

²Dermatology, Osaka City University Graduate School of Medicine

Aim: Communication between cells and the extracellular matrix (ECM) impacts the regulation of various cellular functions. The focal contacts (FCs) are highly dynamic adhesive devices in mesenchymal cells, while hemidesmosomes (HDs) had been believed to be stable anchoring structure in epithelial cells. However, recent studies showed that HDs are also dynamic. In addition to HDs, FCs also have been found to be in keratinocytes. However, there have no detailed studies focused on the relationship between HDs and FCs in stable and/or motile keratinocytes. In order to characterize them, we conducted the live cell imaging of stable and motile keratinocytes.

Methods: We used dynamics of HDs probed with YFP-tagged beta4 integrin and FCs probed with CFP-tagged actinin-1 under confluent or wound culture conditions in live HaCaT keratinocytes. Moreover, drugs which affect cytoskeletons for keratinocytes (cytochalasin D) were added onto the same culture conditions.

Results: When HaCaT cells migrate, FCs firstly appeared at the leading edge of motile HaCaT cells and next matured. Then HDs filled into the "FC-rich" region. FCs of the leading edge in motile live HaCaT cells disappear after HDs which filled into the "FC-rich" matured in that region. And then, new FCs assembled again in next new region and same circuit was repeated. When FCs in live motile HaCaT cells in the presence of cytochalasin D (200 nM) were disrupted, dynamics of HDs were completely stopped moving and not assemble new. By the above, mature FCs formation leads HDs to assemble and move.

34

Development and evaluation of matrix for autologous cultured dermal substitute acceptable for simultaneous transplantation with auto-skin graft

Yasuhiro Matsumoto, Shintaro Yamada, and Yoshimitsu Kuroyanagi
R&D Center for Artificial Skin, Allied Health Science, Kitasato University

Aim: Autologous cultured dermal substitute (CDS) is successfully applied on a full-thickness skin defect after removal of burn scar in order to prepare a wound bed. The next strategy is to apply autologous CDS, followed by grafting split-thickness auto-skin at one time operation. This study is aimed to investigate the take of auto-skin graft.

Method: The matrix composed of hyaluronic acid (HA) sponge and collagen (Col) gel was prepared (group I). CDS was fabricated with HA sponge and Col gel containing human fibroblasts at a density of 4×10^5 cells/cm² (group II). Commercially available artificial dermis composed of atelo-collagen was used as a control. Each material was applied to a full-thickness skin defect (4×3 cm) in dorsal region of F344 nude rat in deficiency of T cell function. At the same time, an excised skin (4×3 cm) was transplanted on each material. The engraftment of auto-skin was evaluated 2 weeks later.

Results: The region showing normal physiological property and skin color tone was defined as a take area. Discolored region was defined as a necrotic area. Graft survival was evaluated by calculating the ratio of both areas. The take rate of control, group I, and group II was $28.6 \pm 12.2\%$, $56.8 \pm 21.8\%$, and $60.1 \pm 9.0\%$, respectively. Group II was more favorable, compared with control.

Conclusion: The matrix of CDS needs to provide with a prompt nutrient supply from wound bed to skin graft. The matrix composed of HA sponge and Col gel appeared to be acceptable for the simultaneous transplantation with auto-skin graft.

GENERAL SESSION 3: TISSUE REGENERATION

19

Fragmin/protamine microparticles as a carrier for protection of FGF-2 biological activity and controlled release tool of FGF-2 for neovascularization *in vivo*

Shingo Nakamura¹, Satoko Kishimoto², Masaki Nambu³, Yasuhiro Kanatani², Takamitsu Ishizuka¹, Hidemi Hattori², Bonpei Takase², Tomoharu Kiyosawa³, Tadaaki Maehara¹, Masayuki Ishihara²

¹Dept. of Surg.,

²Res. Instit.,

³Dept. of Plast. Reconstr. Surg., National Defense Medical College, Tokorozawa, Saitama, Japan

Aim: We investigated the capability of fragmin/protamine microparticles (F/P MPs) as a carrier to protect the activity of FGF-2 from inactivation, and introduce neovascularization *in vivo*.

Methods: Mixing of low-molecular-weight heparin (fragmin) with protamine resulted in water-insoluble microparticles of about 0.5–1 µm in diameter. For investigation of protection effect on the activity of FGF-2 in inactivated condition, FGF-2-containing F/P MPs was heated or trypsinized. Those solutions were used for the culture of human dermal microvascular endothelial cells (HMVECs; FGF-2 is essential for its growth). To evaluate the activity of FGF-2, the growth rate of HMVECs was determined. For investigation of neovascularization induced by FGF-2-containing F/P MPs, the solution of FGF-2-containing F/P MPs was injected into the back subcutis of mice. The mice were sacrificed and tissues were dissected to evaluate neovascularization.

Results: By interaction with F/P MPs, the biological half-life of FGF-2 was prolonged and protected from inactivation caused by heat or proteolysis. After subcutaneous injection of FGF-2-containing F/P MPs into the mice, significant neovascularization and fibrous tissue formation were observed near the site of injection.

Conclusions: These results indicate that F/P MPs are used as a controlled release carrier of FGF-2 and may be powerful tool for therapy in ischemic disease.

20

Aggregation and neovascularization effect of adipose tissue-derived stromal cells with fragmin/protamine microparticles

Satoko Kishimoto^{1,4}, Shingo Nakamura², Masaki Nambu³, Yasuhiro Kanatani¹, Hidemi Hattori¹, Bonpei Takase¹, Tadaaki Maehara², Tomoharu Kiyosawa³, Masahiro Tagawa⁴, Masayuki Ishihara¹

¹Research Institute, National Defense Medical College, Saitama, Japan

²Dep. Surgery, National Defense Medical College, Saitama, Japan

³Dep. Plastic and Reconstructive Surgery, National Defense Medical College, Saitama, Japan

⁴Graduate School of Nippon Veterinary Medicine and Life Science University, Tokyo, Japan

Aim: We prepared fragmin/protamine microparticles (F/P MPs) as cell carriers in cell transplantation studies. F/P MPs consist of insoluble particles (about 1–0.5 µm in diameter). These adhered with the surface of various cells and formed cells / F/P MPs aggregates. In our study, we examined the capability of F/P MPs to enhance the viabilities of various cells and the potential to use as cell carriers *in vivo*.

Method: We made adipose tissue-derived stromal cells (ATSCs) and F/P MPs aggregates and evaluated cell protective effect in suspension culture with MTT assay. Furthermore, we performed administration of this aggregate in the back of nude mice and evaluated neovascularization effect histopathologically.

Result: F/P MPs adhered with ATSCs with in 30 minute and formed ATSCs and F/P MPs aggregates in suspension culture for about 1 hour and maintained viability for at least 3 days. In addition, neovascularization and granulation tissue formation were induced significantly near the site of injection in comparison with ATSCs alone.

Conclusions: We demonstrated that the cells and F/P MPs aggregates were useful as cell carriers. In particular, this study suggested the possibility that ATSCs and F/P MPs aggregates could be applied to a treatment of ischemic disorders.

GENERAL SESSION 4: OTHER TOPICS

26

Fractional photothermolysis for the treatment of post operative cleft lip scar

Tamada I., Nakajima T.*, Mori A.**, Sakamoto Y.*, Yamazaki S.*
Dept. of Plastic and Reconstructive Surgery, Tokyo Metropolitan Kiyose Children's Hospital, *Dept. of Plastic and Reconstructive Surgery, Keio University School of Medicine,
**Clinic Mori

Aim: One of the most important subjects concerning cleft lip repair is post operative scar of the white lip. Surgical correction may be considered but in many cases are hesitated because of long down time. To improve the scar, many treatment modalities were introduced before, for example ablative resurfacing, non-ablative skin tightening, iontophoresis, massage cream and so on. But they do not always bring satisfactory results. In this study, we used the newly presented laser instrument using fractional photothermolysis theory and investigated its validity.

Methods: The laser instrument we used in this study was Lux 1540TM hand-piece of StarLux[®] system which irradiates erbium glass laser of 1540 nm wavelength. Treatments were made by one pass of 100 micro beam(mB)/cm² at fluence of 45mj, pulse width of 15msec. Fifteen patients received total more than three times treatment. One month after the third (in one patient, the fourth) treatment, outcomes were evaluated for degree of improvement by both the patients' (or their families') and the doctor's visual impression (dramatic improvement, moderate improvement, mild improvement, no improvement, worsened). In some patients, magnified photographs were taken by facial measurement instrument, Robo Skin Analyzer for further observation.

Results: In many cases, improvement of the scar appearance was obtained. The face measuring instrument indicated improvement of the scar unevenness and distinctness.

Conclusions: Treatment using fractional photothermolysis demanded for no down time and brought a certain improvement, and was thought to be one of the important treatment strategies in post operative cleft lip scars.

Reference:

Manstein D, Herron GS, Sink RK, Tanner H, Anderson RR: Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. *Lasers Surg Med.*, 34: 426–38, 2004

GENERAL SESSION 5: ULCER

27

Contribution of quorum sensing to the development of biofilm and virulence of *Pseudomonas aeruginosa* in rat wound infection

Lianbo Zhang¹, Masahiro Tachi¹, Emi Kanno², Souhati Toriyabe¹, Masahiro Kosaka¹

¹Department of Plastic and Reconstructive Surgery, Tohoku University Graduate School of Medicine

²Tohoku University School of Health Sciences

Aim: To evaluate the role of quorum sensing on the wound healing and biofilm formation of *P. aeruginosa* wound infection, the *in vivo* pathogenic effects of the wild-type *P. aeruginosa* PAO1 and its double mutant.

Methods: A total 42 full thickness skin wounds were created on rats, the wounds were randomly assigned into three groups. Wounds were inoculated with suspension of wild-type *P. aeruginosa*, a double mutant (*lasI/rhlI*) and without inoculation of bacteria as a blank control. On the day1, day3, day7 and day10 after operation, the following parameters of the *P. aeruginosa* infection were examined: (i) spread of the *P. aeruginosa* strain within the wound skin, ii ratio of wound epithelization, iii biofilm formation analyzed by fluorescence microscope.

Results: In comparison with wild-type PAO1, biofilm formation of *lasI/rhlI* mutants was reduced on day7; on day7 and day10 post-infection wounds with mutant PAO, showed great wound reduction. In contrast, there was no significant difference between the numbers of bacteria in the tissues on the indicated day, that may be due to the small numbers of the subjects.

Conclusions: These results suggest that the *lasI* and *rhlI* genes of *P. aeruginosa* play an important role in the spread of *P. aeruginosa* within wound skin and development of biofilms *in vivo*.

28

The strategies for the leg skin ulcer due to vascular disorder using the evaluation of skin perfusion pressure

Hiroshi Yasuda*, Jyunko Yasuda*, Yujin Doi*, Yousuke Nishimura**, Takashi Kajihara**, Hiroyoshi Yokoi***

*Department of plastic and reconstructive surgery, University of Occupational and Environmental Health,

**Department of cardiovascular surgery, University of Occupational and Environmental Health,

***Department of cardiology, Kokura Memorial Hospital

Aim and Methods: Leg skin ulcers due to cardiovascular disorder and /or diabetes are increasing. For their treatment, evaluation of local blood circulation is very important. There are many tools for evaluation of local blood circulation. Among of them, skin perfusion pressure (SPP) is very useful. Using this tool, strategies for skin ulcer treatment are 1) If SPP level is above 40mmHg, treatment for skin ulcer should be done immediately, 2) If SPP level is below 40mmHg, angioplasty should be performed before skin ulcer treatment, 3) If angioplasty could not performed, skin ulcer should be dry condition for waiting or amputation above or below knee level should be done. Following these strategies, we reported four treated cases of the leg ulcers after evaluation of SPP.

Results: In case 1, SPP showed low level, we perform the angioplasty (vascular bypass) before ulcer treatment. After the SPP level turned to high, skin graft was performed and got good result. In case 2, SPP level showed good, the ulcer was healed with conservative therapy. In case 3, SPP level was very low and angioplasty was impossible because of collagen disease, we could not treat the skin ulcer. In case 4, SPP was same as case3, amputation with thigh level was performed.

Conclusions: Before using SPP, we confused the strategy for the leg ulcer treatment. But after using this tool, we can easily plan the strategy. SPP is very useful tool for evaluation local blood circulation.

32

The effect of aspartyl proteinase from *Candida albicans* in duodenal ulcer induced by cysteamine in rats

Longxue Jin¹, Masashi Yoshida², Yuko Kitagawa¹, Tetsuya Nakamura¹, Hideki Ishikawa³, Go Wakabayashi⁴, Minoru Tanabe¹, Shigeyuki Kawachi¹, Masahiro Shinoda¹, Tetsuro Kubota⁵, Yoshiro Saikawa¹, Norihito Wada¹, and Masaki Kitajima²

¹Department of Surgery,

⁵Division of Comprehensive and Advanced Medicine,

²Keio University School of Medicine, Center for Digestive Disease, Mita Hospital, International University of Health and Welfare,

³Division of Surgery, Eiju General Hospital

⁴Department of Surgery I, Iwate University School of Medicine

Address: Department of Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582, Japan.

Background and purpose: It was reported that the cure rate of *Candida* positive peptic ulcer was low as 30% in six months. *Candida* infection not only aggravated the duodenal ulcer but also delayed the wound healing of the duodenal ulcer in cysteamine-induced duodenal ulcer rats in the previous studies. Aspartyl proteinases from *Candida* were reported as key factors in *Candida* infection. The purpose of the present study was to clarify the effect of aspartyl proteinases in duodenal ulcer with *Candida* infection by administration of pepstatin A, an aspartyl proteinase inhibitor.

Methods: All rats were administrated with cysteamine after anesthetization on Day 1. *C. albicans* and saline was administrated to the rats in control group (n = 8) from Day 3 to 6, and *C. albicans* and pepstatin A were administrated to the rats in the pepstatin group (n = 8). The rats were killed on Day 7. The depth and area of duodenal ulcer were assessed between the two groups. The immunohistochemistry for vascular endothelial growth factor A (VEGF-A) and proliferating cell nuclear antigen (PCNA) was performed.

Results: On Day 7, the prevalence of duodenal ulcer in the control group and the pepstatin group were 75.0%, 25.0%, respectively ($P < 0.05$). The duodenal ulcer area in the control group and in the pepstatin group was 7.93 mm², 0.58 mm², respectively. There were significant differences on duodenal ulcer area between the two groups ($P < 0.05$). However, there was not significant difference on the numbers of the VEGF-A positive cell and PCNA positive cell between the control group and the pepstatin group.

Conclusion: It was suggested that the wound healing of duodenal ulcers was affected by aspartyl proteinase from *Candida* in cysteamine-induced duodenal ulcer with *Candida* infection.

SYMPOSIUM 1: LEADING-EDGE TREATMENT FOR DECUBITUS

S1-01

The clinical characteristics and future problems of the treatment of pressure ulcers in patients with type II diabetes mellitus

Kaneyuki Matsuo

Matsuo Kenkou Clinic, Sendai, Japan

Aim: The purpose of this study is to assess the clinical characteristics and future problems associated with pressure ulcers in home-cared patients, complicated with type II diabetes mellitus.

Patients and Methods: We investigated twenty pressure ulcers in home-cared patients with type II diabetes mellitus from 2001 to 2006.

Results: It took 31 weeks that pressure ulcers in home-cared patients with type II diabetes mellitus covered with normal epithelial cells over three-fourth. On the other hand, those without diabetes mellitus healed in 27weeks ($p < 0.05$). There were no statistically significant correlations to gender, age and the home-cared duration with the healing time of pressure ulcers between the diabetic and non-diabetic groups. The cases that showed progressive histological invasion, lymphocytopenia, severe local infection and over 7.5% of glycohemoglobinA1c revealed a longer healing time. Early induction of insulin therapy for home-cared patients with diabetes mellitus was significantly correlated with good response for pressure ulcers.

Conclusion: Early treatment for pressure ulcers, under 7.5% of glycohemoglobinA1c, early induction of insulin therapy, no lymphocytopenia and no local infection were significant predictive factors of good healing response for pressure ulcers in home-cared patients with diabetes mellitus. These factors were related to local blood flow, immune response and metabolic disorders.

S1-02

Treatment of osteomyelitis underlying pressure ulcers

Hiroaki Oka, Mika Shinoyama, Katsuyuki Urushihara, Takahiko Moriguchi
Department of Plastic and Reconstructive Surgery, Kawasaki Medical School, Kurashiki city, 701-0192 Japan

Aim: A guideline for the local treatment of pressure ulcers was published by The Japanese Society of Pressure Ulcers in 2005 with treatment being based on a DESIGN evaluation. We introduce a part of the guideline and describes a case of osteomyelitis underlying pressure ulcers.

Methods: Fifty pressure ulcers in 31 men and 19 women with a mean age at presentation of 60 years (32 to 82 years) were reconstructed between January 2000 and August 2007. In this series, there were 20 sacral, 20 ischial, and 10 trochanteric pressure ulcers. A diagnosis of osteomyelitis was also made in the case of eight pressure ulcers. Those patients who were diagnosed with osteomyelitis before reconstruction, first underwent treatment with parenteral antibiotics for two to three weeks. Then we performed high pressure pulse lavage for four to six weeks. After these treatment, muscle or fascial flap closure of the overlying pressure ulcers were performed.

Results: In 21 case, suture dehiscence was caused by hematoma, seroma, and infection. In eight of these cases, the wounds were resutured. In four of the eight cases, osteomyelitis underlying pressure ulcers was observed.

Conclusions: Our protocol for osteomyelitis underlying the pressure ulcers might be effective since Han¹⁾ reported that in 15 cases of osteomyelitis underlying pressure ulcers, complications were observed in all but one. However the validity of this treatment must be verified in additional cases in the future.

References:

Hans H, Lewis Jr VL, Wiedrich TA et al.: The value of Jamshidi core needle bone in predicting postoperative osteomyelitis in grade 4 pressure ulcer patients. *Plast Reconstr Surg.*, 110: 118-122, 2002

S1-03

The treatment of infected pressure ulcers with osteomyelitis

KUNIO TSUKADA

Takaoka Ekinan Clinic, Takaoka City, Toyama, Japan

We had two cases of infected pressure ulcers, those were treated very effectively with intravenous injection of antibiotics. Both had osteomyelitis detected by x ray.

The first case was an 82-year-old male. He had an infected sacral pressure ulcer. During the treatment, his wound was fully covered by granulation tissue. But he still complained of very sharp pain on the wound and a lot of bloody exudate came from the wound. A CT scan showed dissolution of sacral lamina. He was

Abstracts

injected PIPC antibiotics intravenously for two weeks. The severe sharp pain and bloody exudate disappeared immediately, as the wound started to heal quickly.

The second case was an 87-year-old female. On her second toe, she had a pressure ulcer covered by granulation tissue. She complained of severe sharp pain and the wound drained a lot of bloody exudate. Radiograms showed bone dissolution in the pressure ulcer. Sensitive CEZ antibiotics were given intravenously to her for two weeks. That was very effective, and her pain receded and the wound healed up.

Non-healing pressure ulcers, with severe, sharp pain and bloody exudate, should be examined with radiograms or CT scans to diagnose if they are accompanied by osteomyelitis. In case of that, intravenous injection of effective antibiotics for two weeks is recommended.

S1-05

New material for wrap therapy thin teflon film with small hole

Tadakazu Kubo (pharmacist), Atsushi Tanaka (M.D)
Non-profit-organization Bedsores Association, Kamogawa City Chiba Japan

Aim: Wrap therapy is very typical way for pressure ulcers. So we study which chemical material is more effective, by its ability of holding protein, repelling water, promeric, friction, holding static electricity etc.

Methods: I examined chemical and physical character of each material. Especially I study which material can maintain basic FGF in wound.

Results: I found thin Teflon film with small hole is most effective by physically and chemically experiences, also by clinical experiences.

S1-06

Reconsideration of povidone-iodine-sugar

Tadakazu Kubo (pharmacist), Atsushi Tanaka (M.D)
Non-profit-organization Bedsores Association

Aims: Povidone-Iodine(PVP-I) with sugar has been typical medicine for pressure ulcers in Japan. This medicine is depend on the function of PVP-I to kill on contact a wide variety of bacteria etc. PVP-I is stable chemical complex of polyvinylpyrrolidone(PVP) and elemental iodine. But it is said that PVP-I medicine causes trouble for a human's cells, so we need a safer form.

Method: I examined the chemical property of PVP-I and also examined it by speed of disappearance, comparing among PVP-I sugar medicines and cadexomer Iodine medicines.

Result: Chemical complex of PVP-I is influenced by sodium ion in buffer solution but adding potassium iodide in make it rather stable but different form from original PVP-I. This form might lost original property, which is releasing iodine gradually in water and killing bacteria speedy, of PVP-I. Then, I choose special form which PVP-I solution is in oil in order to make safety to cell and this form has long term of its life like cadexomer iodine. We tested it on 60patients and found it more effective than normal products.

Conclusion: We make this medicine more safety with killing bacteria more selectively because oil can protect human cell and cytokine. Because, considering mechanism of reaction of iodine, there might be side effect to human cell originally. Additionally, it prevents skin from drying and is a very useful form.

SYMPOSIUM 2: WOUND HEALING AND INFECTION CONTROL (PREVENTION AND TREATMENT OF WOUND INFECTION)

S2-01

Bacterial infection in patients with foot ulcers

Toru Hiyoshi, Syuhei Morimoto and Sumihisa Imakado
Division of Wound care center, Japanese Red Cross Medical Center

Objectives: To analyze frequency of bacterial infection in newcomer out patients case in chronic foot ulcerative lesion in our wound care center, and to ascertain the days for wound healing.

Methods: Screening of medical records 105 cases (male 77, female 38) of chronic foot wounds in our wound care center who were visited from December 2005 to October 2007. We evaluated for wounds stage (NPUAP classification), cause of disease, and co-incidence of diabetes, hemodialysis.

Results: Cause of foot ulcerative lesion PAD (Peripheral arterial disease) alone 20%, DM (Diabetes mellitus) alone 29%, PAD+DM 25%, Venous congestion 14%, and others 17%. Wound stage III or IV cases were 62 cases (59%). Patients who has diabetes mellitus were 67 cases (63.8%), and hemodialysis 40 cases (38.1%). Bacteriological examination were performed 39 cases (36%).

Single infection is 17 cases (45.6%) and multiple infection is 19 cases (48.7%). *Staphylococci* were most common isolate in our center (41%), including MRSA(11%). *Coryne bacterium spp.*(11%), *Streptococci* (7%) and *Candida albicans* (7%) were the next group. Wound healing days longer in the groups of isolate MRSA than other SA (135.8 vs 84.3 days n.s.) and visiting frequency of the clinic until wound healing was significant difference in the each group (15.8 vs 8.3 p < 0.05). There were no significant differences of these parameter in the group of single isolate or multiple isolate, and group with diabetes or without diabetes.

Conclusion: Microorganisms isolated from chronic foot wounds were various kinds of species but SA is most common. Duration of wound healing was prolonged by MRSA infection.

S2-02

Prevention of surgical site infection following gastric surgery – focus on incisional SSI

Hitoshi Kanno, Teruo Kiyama, Takeshi Okuda, Itsuo Fujita, Syunji Kato, Toshiro Yoshiyuki, and Takashi Tajiri
Surgery for Organ Function and Biology Regulation, Nippon Medical School Graduate School of Medicine, Tokyo, Japan

Aim: Surgical site infection (SSI) is a potentially morbid and costly complication following major gastric surgery. We close the abdominal wall of patients in unified way regardless of the surgical procedure used as described below. 1. continuous suture of peritoneum by 0-PDSII, 2. continuous suture of membrane of muscle by 0-PDSII, 3. lavage of wound, 4. continuous buried suture of derma, 5.dressing epidermides with steri tape.

Methods: We conducted retrospective SSI surveillance of all gastric resection in a single institution from January 2004 to December 2007. The out come of interest was a diagnosis of incisional SSI as defined by the Center of Disease Control and Prevention. The incidence of SSI in this study was compared with the rates of incisional SSI in this patient population reported by the Japanese Nosocomial Infection Surveillance (JNIS).

Results: The incidence of SSI accounted for 6.41%, and it was less than JNIS (9.59%). The incidence rates of incisional SSI accounted for 3.21%. The incidence rates of SSI in laparoscopic and open surgery accounted for 2.27% and 9.00%, respectively, and both of them were less than JNIS (3.45% and 9.97%, respectively).

Conclusions: In this study, our surveillance data was better than the national average. We would take an extra effort to reduce the incidence rates of SSI, and get it under control by not only surgical technique but also instruments.

S2-04

Results of SSI surveillance at our institute

Masanori Watanabe, Hidetsugu Hanawa, Masaki Koizumi, Tetsutaka Toyoda, Satoshi Nomura, Kentaro Maejima, Osamu Komine, Satoshi Mizutani, Masanori Yoshino, Hideki Bo, Akira Tokunaga, Takashi Tajiri*
Institute of Gastroenterology, Nippon Medical School Musashikosugi Hospital
*Department of Surgery, Nippon Medical School

Aim: Our institute started surgical site infection (SSI) surveillance after digestive surgery and various measures based on the CDC guidelines in July 2005. We investigated changes in the incidence of SSI over time before and after the start of surveillance.

Methods: We surveyed the results of 1417 cases of digestive surgery, consisting of a group of 263 cases before the start of surveillance and a group of 1154 cases after the start of surveillance, and investigated the changes over time in each group.

Results: The incidence of SSI was 8.0% before the start of surveillance as opposed to 5.3–6.8% after the start of surveillance. It obviously decreased after the start of surveillance. According to organ, the greatest improvement was seen after surgery on the rectum and appendix. The average incidence of SSI during the 2 years after the start of surveillance was esophagus, 25.0%; stomach, 7.4%; colon, 10.3%; rectum, 16.9%; liver and pancreas, 15.5%; appendix, 3.3%; and comparable to JNIS's national average.

Conclusions: It is necessary to take measures to lower the incidence of SSI in organs with high rates and after frequently performed operations. It is important not only to perform SSI surveillance but also to obtain feedback and take counter measures.

SYMPOSIUM 3: NEW WOUND MANAGEMENT (INCLUDING SURGICAL SUTURE MATERIAL)

S3-01

Treatment for nail bed defects using artificial dermis and B-FGF

Akira Sugamata, Naoki Yoshizawa, Yasuko Matuoka, Kenich Yamaguchi
Department of Plastic Surgery, Tokyo Medical University Hachioji Medical Center, Tatemachi 1163, Hachioji city, Tokyo

Aim: Nine cases of nail bed defects were treated using artificial dermis and b-FGF. The method was then evaluated in terms of efficiency of outcomes.

Methods: At the primary treatment, artificial dermis was applied after cleaning of the wounds. The silicone membrane of artificial dermis was removed 3~5 days after primary treatment, and b-FGF was applied every 2 days until wounds closed. In cases of avulsion and amputation at the level of the nail matrix, artificial dermis was used after reconstruction of the palmer soft tissues flaps, and b-FGF was applied as the same method. The flaps used were two reversed finger arterial flaps and one advanced neurovascular finger flap.

Results: The nail plates regenerated in all cases of nail bed defect. The nail plates grew normally in cases of small nail bed defect, but in cases of relatively large nail bed defect, the deformities and shortenings of nails were observed. Even in cases of avulsion and amputation at the level of the nail matrix, if the nail matrix was preserved, the nail plates were regenerated combined with reconstruction of finger tip soft tissues using arterial palmer flaps of the finger.

Conclusion: We concluded that this method is very useful in reconstruction of nail bed injuries to salvage the nail plates.

S3-03

The effectiveness of a contrived way of using fiber products to treat wounds

Takeru Mashima, Tomohisa Shimokobe, Ei Sasaki, Haruto Oe, Shuzou Amano, Junko Takane, Mamoru Ariyoshi, Tsuyoshi Narisada
Tobata Rehabilitation Hospital
Kensei Nagata
Orthopedics surgery of Kurume University

Introduction: We found a new way of using fiber products, Kaltostat (Convatec) and Beschitin (Unitika), and tried to treat some kinds of wounds.

Materials and Methods: A newly-made tape (NMT) was prepared by spreading fiber products over an adhesive of atransparent film dressing, Opsite (Smith&Nephew). Occlusive dressing therapy (ODT) using NMT with some ointments, Fiblast spray (Kaken), Prostandine cream (Ono), and White petrolatum (Ebisu) were performed in the treatment of a scar formation of untreated wound, cracked heels, some pressure ulcers and some post-operative wounds, with daily dressing change for about three weeks.

Results: The scar formation of untreated wound showed a decrease in redness, and the other wounds were healed completely.

Discussion: NMT was useful to accomplish ODT, which enable us to treat the wounds easily and steady.

S3-07

Surgical site infection in patients with radical cystectomy

Satoshi Takahashi, Yuki Kyoda, Naoki Itoh, Taiji Tsukamoto
Department of Urology, Sapporo Medical University School of Medicine, Sapporo, Japan

Aim: In the field of urology, radical cystectomy (RC) with urinary diversion or reconstruction can be classified as a "contaminated operation" and the incidence of surgical site infection (SSI) in RC is higher than in other types of urologic surgery. Therefore, we tried to reduce the incidence of SSI in RC by modification of perioperative procedures.

Methods: The subjects were patients with RC with urinary diversion or reconstruction from 1994 to 2006. Until the year 2000, we disinfected the surgical site and changed the gauze daily until removal of sutures. From 2001 to 2003, we covered the surgical site with dressing material until removal of sutures without a disinfectant. After 2004, we covered the surgical site with a dressing for 2 days postoperation, after which it was removed.

Results: The incidences of SSI were 29.2% (19/65) from 1996 to 2000, 36.6% (15/41) from 2001 to 2003 and 16.5% (13/79) after 2004. There were significant differences between the former 2 groups and the latter one ($p = 0.0158$).

Conclusions: The incidence of SSI in RC with urinary diversion or reconstruction has been decreasing recently; however, it is still relatively high. Early removal of the dressing could contribute to the prevention of SSI.

S3-08

The effect of photo-crosslinkable chitosan hydrogel containing medium on full-thickness skin defects after deep dermal burn

Yasuhiro Kanatani, Masayuki Ishihara, and Tetsuro Kiyozumi
Research Institute, National Defense Medical College, Tokoroawa, Saitama, Japan

Aim: The current study was designed to determine the effect of novel photo-crosslinkable chitosan hydrogel containing medium (medium-Az-CH-LA) on deep dermal burn.

Methods: Sixteen male Wistar rats were randomly divided into two groups that were treated with medium-Az-CH-LA ($n = 5$) or a collagen sponge ($n = 5$). Under anesthesia, the dorsal fur was shaved and the skin was exposed to water at 95 degrees C for 10s. After 2h, damaged tissue was removed from the fascia and dressed with medium-Az-CH-LA or a collagen sponge.

Results: Histological examination revealed that the thickness of the granulation tissue in the medium-Az-CH-LA-treated group was greater than that in the collagen sponge-treated group. Moreover, degradation and neovascularization occurred earlier in the group treated with medium-Az-CH-LA compared with the collagen sponge-treated group.

Conclusions: These findings suggest that early degradative and angiogenic activities of medium-Az-CH-LA may be beneficial for granulation tissue formation in deep dermal burn wounds.

References:

1. Kiyozumi, T. et al., J Biomed Res. B Appl. Biomater. (2006) 79, 129-136
2. Kiyozumi, T. et al., BURNS (2007) 33, 642-648

SYMPOSIUM 4: SCAR PREVENTION AND TREATMENT

S4-02

Analysis of the effect of early treatment for postoperative scar to prevent keloid

Tosa M¹, Murakami M¹, Hyakusoku H²

¹Department of Plastic and Reconstructive Surgery, Nippon Medical School Musashikosugi Hospital, Kawasaki, Japan

²Department of Plastic and Reconstructive Surgery, Nippon Medical School Hospital, Tokyo, Japan

Aim: The cause of keloid is not clear, therefore, it is important to attempt to prevent and treat keloid in the early stage. The lower abdominal region is known as a site in which keloid is easy to develop. Since May 2006, we have started an outpatient clinic which examines a postoperative scar developed after an obstetrics and gynecology operation. The aim of this study was to clarify the time when the postoperative scar began to transform into keloid and when we should begin treatment to prevent keloid.

Material and Methods: We reviewed patients who had an obstetrics and gynecology operation between May 2006 to May 2007 with reference to their postoperative scar. We examined the site of operation postoperatively at 7 days, 1 month, 3 months, and 6 months to see whether a scar was arising. For the patients who had scar arising at 1 month, we started treatment with steroid tape and compared the incidence of keloid formation with that of the untreated patients.

Results: We could classify the scars into 3 groups at 1 month postoperatively; i. mature cicatrix, ii. hypertrophic scar, and iii. intermediary between A and B. The incidence of keloid was 18.3% in the treated group versus 31.2% in the untreated group.

Conclusion: It was concluded that it is important to check for a cicatrix at 1 month postoperatively and begin therapy to prevent keloid.

S4-03

Evaluation of risk factors for effect of therapy in keloid and hypertrophic scar

Tosa M¹, Murakami M¹, Hyakusoku H²

¹Department of Plastic and Reconstructive Surgery, Nippon Medical School Musashikosugi Hospital, Kawasaki, Japan

²Department of Plastic and Reconstructive Surgery, Nippon Medical School Hospital, Tokyo, Japan

Aim: There is no established classification for keloid and hypertrophic scar. Establishment of a classification system that enables a prognostic prediction and choice of therapy for keloid and hypertrophic scar is highly desirable. We considered what types of keloid and hypertrophic scar were intractable and aimed to clarify risk factors of the effect of therapy.

Abstracts

Material and Methods: From patients with keloid and hypertrophic scar treated at our hospitals between 2000 to 2005, we extracted cases that showed a poor response even after more than two years of treatment or recurred after surgery and postoperative electron beam therapy. We analyzed clinical and histopathological data as well as epidemiological data of these cases.

Results: Common findings among the cases were that they had multiple lesions and a persistence of lesion more than ten years and an infected wound in keloid and hypertrophic scar.

Conclusion: It was concluded that a keloid or hypertrophic scar with frequent recurrence, long persistence, and infection is difficult to be managed successfully.

SYMPOSIUM 5: UPDATE REGENERATION MEDICINE (STEM CELL – MOLECULAR BIOLOGICAL APPROACH) S5-01

Skin regeneration using skin derived precursors in mice

Kazuo Kishi, Ruka Ninomiya, Keisuke Okabe, Hideo Nakajima, and Tatsuo Nakajima
Department of Plastic Surgery School of Medicine Keio University 35
Shinanomachi Shinjuku Tokyo Japan

Aim: Skin derived precursors (skps) can be cultured from the dermis of embryonic day (E) 17 mice. Although skps have been shown to possess multipotency, the ability to induce hair follicle has not been shown. In the present study, we showed whether the skps have the ability to induce hair regeneration or not using the co-transplantation model on to the scid mice.

Methods: Skps were cultured from the dermis of E17 C57bl/6J mice. Dermis were enzymatically dissociated and filtered through 40 µm filter. After culturing, skps were examined neuronal and adiposal differentiation. Also, the markers of skps, Sca-1, nestin, and fibronectin expressions were examined. Then skps were co-transplanted with the enzymatically dissociated epidermal cells of E17 to the full thickness skin defects of scid mice. Four weeks later, the co-transplanted area was examined macroscopically and histologically. Then the differentiation of the hair was examined immunohistochemically.

Results: Cultured cell aggregations can be cultured from E17 dermis. Cultured cell aggregations can be differentiated into neurons, adipocytes, and expressed markers of skps. In the co-transplantation study, black hairs were regenerated in the transplanted area. Histological sections indicate that hair and sebaceous glands were regenerated completely. Compared to the normal hairs, differential markers were identical immunohistochemically.

Conclusions: Skps possess an ability to induce hair regeneration. Although fetal epidermal cells are needed in the co-transplantation, skps is thought to be very promising cells in skin regeneration.

S5-02

New acellular scalp dermis to make ex-vivo produced skin equivalent with hair follicles

Terashi H., Yokoo S., Sakakibara S., Hashikawa K., Kurata S., Sunagawa T., Makiguchi T., Fujisato T., Itami S., Hasegawa M., and Tahara S.
Departments of Plastic Surgery and Oral Maxillofacial Surgery, Kobe University, Kobe, Japan

Background: Ex-vivo produced oral mucosa or skin equivalent is generated in a serum-free culture system, without the use of an irradiated xenogenic feeder layer, by seeding human keratinocytes onto a human cadaveric dermal equivalent, AlloDerm. However, there is no composite skin with hairs so far.

Aim: The aim of this study is to move one step closer to make tissue-engineered skin equivalent with hair follicles.

Methods: We made new acellular dermis with hair pores chemically and physically from human cadaveric scalp skin.

Results: The new acellular scalp dermis included hair pores and kept the original collagen structures. Immunohistochemical stainings of basement membrane show that laminin 1,5 and type IV, VII collagen are positive.

Conclusions: The new acellular scalp skin with hair pores from human cadaver should be ideal scaffold for future regenerative medicine of baldness. Next, we will culture the hair follicle keratinocytes onto the new equivalent.

S5-04

Accelerating wound healing and improving scar quality by mesenchymal stem cells

Akita S, Akino K, Hirano A
Nagasaki University, Nagasaki Japan

Aim: Ultimate goal of wound healing is obtain better scars. In fact, there are higher evidences of hypertrophic scars after delayed wound healing. We pay special attention to mesenchymal stem cells (MSCs) from early stage of wound to scar forming phase.

Methods: The human MSCs, basic fibroblasts growth factor (bFGF) lipid mediators, keloid-derived fibroblasts are used both in vitro and in vivo studies.

Results: The hMSCs are well-regulated in terms of cell cycle, proliferation and expression of the gene products by bFGF and differentiated toward fibroblast-like cells by a lipid mediator. Co-culture of hMSCs and keloid-derived fibroblasts accelerated cell migration and increased extra-cellular metrical production with ultra-microstructure of micro-villi formation on the cell surfaces. In vivo models, there are epidermal transition by hMSCs with bFGF in an excision and irradiated wounds.

Conclusions: The hMSCs may contribute to accelerated wound healing and better scar formation both in vitro and in vivo.

References:

1. Akino K, Akita S, Yakabe A, Minoda T, Hayashi T, Hirano A. Human mesenchymal stem cells may involve in keloid pathogenesis. *Int J Dermatol.*, in press.

S5-06

Fibroblast growth factor-2 stimulates adipogenic differentiation of human adipose-derived stem cells

Kakudo N, Shimotsuna A, Kusumoto K
Department of Plastic and Reconstructive Surgery, ansai Medical University, Osaka, 570-8507, Japan

Introduction: Adipose-derived stem cells (ASCs) have demonstrated a capacity for differentiating into a variety of lineages, including bone¹, cartilage, or fat, depending on the inducing stimuli and specific growth and factors. It is acknowledged that fibroblast growth factor-2 (FGF-2) promotes chondrogenic and inhibits osteogenic differentiation of ASCs, but thorough investigations of its effects on adipogenic differentiation are lacking.

Material and Methods: In this study, we demonstrate at the cellular and molecular levels in different phases of cell culture leads to a strong enhancement of adipogenesis of ASCs, as induced by an adipogenic hormonal cocktail consisting of 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin, and indomethacin.

Result: FGF-2 significantly enhances the adipogenic differentiation of human ASCs. Furthermore, in cultures receiving FGF-2 before adipogenic induction, mRNA expression of peroxisome proliferator-activated receptor-gamma (PPAR γ), a key transcription factor in adipogenesis, was upregulated.

Conclusion: The results of FGF-2 supplementation suggest the potential applications of FGF-2 and ASCs in adipose tissue regeneration.

References:

1. Kakudo N., Shimotsuna A., Miyake S., Kushida S., Kusumoto K. Bone tissue engineering using human adipose-derived stem cells and honeycomb collagen scaffold: *J Biomed Mater Res A*. 84:191–7, 2008.

SYMPOSIUM 6: CHRONIC WOUND

KEYNOTE PRESENTATION

The molecular biological approach on chronic wound management and strategy for skin regeneration

Ichiro Ono, M.D.
Department of Dermatology, School of Medicine, Sapporo Medical University, Sapporo, Japan

Recent basic research revealed that the normal wound healing process is progressing under the control of the growth factors and the cytokines. On the other hand, the healing of so called chronic wound such as leg ulcers, diabetic ulcers and decubitis has decreased in such responses. So the in many elderly cases, whose general conditions make it impossible to give extensive treatment, a so-called preserved treatment is selected instead of surgical treatments. On the other hand, in recent years, a molecular biology aspect of the wound healing becomes became clear and the growth factors which made of the proliferation can be used actually applied clinically. With respect to the clinical application of substances like growth factors such as bFGF in treatment of so called chronic

wound, defining the methods how to apply them are always important as well as the optimum dose. From our clinical experiences as well as from animal experiences, combined use of growth factors with collagen sponges is very useful strategy for the treatment of chronic wounds. In the present research, we also presented regenerated tissue by the so-called *in situ* tissue regeneration technique. Using this regeneration technique, we tried to regulate the proliferating and differentiating capacity of tissue stem cells present in tissue by transplanting cultured cells, into which morphogen genes have been introduced, as well as the appropriate matrix and cultured skin, and to regulate the series of responses that are involved in the regeneration of skin and its appendages which will be able to apply clinically.

S6-01

Development of wound dressing composed of hyaluronic acid sponge with epidermal growth factor

Mikihito Nakano, Yasuhiro Matsumoto, Yoshimitsu Kuroyanagi
Kitasato Univ. R&D Center for Artificial Skin, Kitasato Sagami-hara 228-8555 Japan

Aim: This study is designed to investigate the efficacy of wound dressing composed of hyaluronic acid (HA) sponge with epidermal growth factor (EGF), focusing on the wound healing in deep dermal burn of rats.

Methods: Two types of wound dressings were prepared. Group 1 is 2-layered sponge composed of cross-linked high-molecular-weight HA and hydrolyzed low-molecular-weight HA. Group 2 is 2-layered sponge composed of cross-linked high-molecular-weight HA and hydrolyzed low-molecular-weight HA including EGF. Control group is a commercially available artificial dermis composed of collagen. The abdomen skin of SD rat was shaved and then contacted with boiling water for 5 sec to result in a deep dermal burn, measuring 35mm in diameter. Three days after burn injury, the necrotic tissue was excised, and covered with each dressing. The wound conditions were observed 7 days after covering with each dressing.

Result: Wound size and granulation tissue formation were evaluated macroscopically and histologically. The wound size decreased in order of group2, group1, and control. There was a slight difference in the granulation tissue formation among these groups.

Conclusion: The wound dressing was designed to allow the first release of EGF and hydrolyzed low-molecular-weight HA, followed by the second release of high-molecular-weight of HA. The results indicate that HA sponge including EGF is a promising wound dressing.

S6-02

Basic FGF induces CASPASE-3 activation and apoptosis in dermal fibroblasts pretreated with TGF- β 1 *in vitro*

Yoshikiyo Akasaka,^a Ichiro Ono,^b Yukio Ishikawa,^a Kinji Ito,^a
Toshiharu Ishii^a

^aDepartment of Pathology, School of Medicine, Toho University, Tokyo, Japan

^bDepartment of Dermatology, Sapporo Medical University School of Medicine, Sapporo, Japan

To clarify the mechanisms undergoing apoptosis in dermal fibroblasts during the course of wound healing, we have examined expression of activated caspase-3 and apoptosis using four granulation tissues-derived fibroblasts (GF-1, 2, 3, 4) and two normal dermal fibroblasts (NF-1, 2). After treated with TGF- β 1 for 48 hours, the GF and NF cells were incubated with bFGF for 48 and 96 hours. GF-1, 2,3 and NF-1 cells exhibited altered morphology, with many cells rounding up and detachment. After staining with propidium iodide and Hoechst reagent, the GF cells exhibited the distinctive morphology of apoptotic cells including nuclear shrinkage and desegregation. To confirm that these observed morphological changes were caused by apoptosis, Western blot analysis was performed and demonstrated that cleaved caspase-3 fragments were detected in the GF-1, 2, 3 and NF-1 cells after treatment of bFGF for 48 and 96 hours. In contrast, almost no cleavage fragments were detectable in the GF-4 and NF-2 cells pretreated with TGF- β 1, irrespective of stimulation of bFGF. Also, cleavage fragments were undetectable in TGF- β 1-pretreated all the cell lines. These results indicate that bFGF can induce apoptosis in the TGF- β 1-pretreated dermal fibroblasts through caspase-3 activation *in vitro*.

S6-03

Effect of acupuncture on lower limb ischemia

Shuji Suzuki^{1,2}, Shigeru Ichioka², Takashi Nakatsuka², Hiroshi Omata³,
Satoru Yamaguchi³ and Toshihide Mimura³

¹Yamanashi University, Department of Dermatology, Division of Plastic Surgery

²Saitama Medical University, Department of Plastic and Reconstructive Surgery

³Saitama Medical University, Department of Oriental Medicine

Aim: To investigate the effect of acupuncture on the lower limb, cutaneous blood flow and oxygenation were measured by skin perfusion pressure (SPP) and transcutaneous partial pressure of oxygen (TcPO₂) at 4 points on the lower limb.

Methods: The trial involved 20 healthy volunteers (10 males and 10 females) averaging 33.8 years old. The Zusanli and Sanyinjiao acupoints of the right leg were stimulated with electro-pulsed acupuncture for 10 minutes at 1 Hz. SPP levels at 4 measurement points before and after acupuncture, and TcPO₂ levels at the same points before, during and after acupuncture were measured. The same trial was conducted for 9 patients with peripheral arterial disease (PAD) averaging 65.1 years old. These PAD patients received once-a-week acupuncture and accompanying measurements for 4 weeks, and the Kidney Disease Quality of Life questionnaire, and Visual Analogue Scale scores of pain and general condition were assessed on the first and last acupuncture days.

Results: Among the healthy adults, SPP increased significantly at the base of the first toe and ankle, and TcPO₂ levels increased significantly at all 4 points. Among the PAD patients, SPP increased significantly at the dorsum of the foot and ankle after the first acupuncture and at all 4 points after the last acupuncture, but TcPO₂ revealed no increase. The Physical Function of SF-36 showed significant increase after the last acupuncture.

Conclusions: Among healthy adults, blood flow increased more in the peripheral areas. In PAD patients, 4 weeks of acupuncture resulted in increased blood flow to most areas of the lower limb.

S6-04

Outcomes of patients with CLI (critical limb ischemia) and chronic leg ulcers treated without revascularization

Yoriko Tsuji¹, Ikuro Kitano¹, Yoshihiko Tsuji¹, Hiroatsu Iwatani¹,
Hiroto Terashi², Shinya Tahara²

¹Wound Treatment Center, Shin-Suma General Hospital

²Department of Plastic Surgery, Kobe University Graduate School of Medicine

Aim: To investigate prognosis of patients with CLI treated without revascularization, we compared outcomes of patients treated with and without revascularization.

Methods: A retrospective review demonstrated 176 CLIs which were treated in the Wound Treatment Center in Shin-Suma General Hospital.

Results: Wound healing rates were 75.6% in the patients treated with revascularization, and 39.2% without revascularization. Major amputation rates were 11%, and 15.7%. Mortality rates were 10%, and 23.5%.

Conclusions: Major amputation continues to result in significant morbidity and mortality. Revascularization is most important for limb salvage, but in case of poor candidates for revascularization, major amputation is avoidable by thorough foot management.

S6-08

Assessment and treatment of diabetic foot ulcer based on skin perfusion pressure

M. Kawai^{1,5}, S. Mihara¹, T. Mizuno¹, N. Madokoro¹, H. Noda¹, Y. Higashi²,
T. Umemura², K. Nishioka², S. Shimose³, K. Yokota³, K. Okada⁴, K. Sato⁴,
T. Sueda⁴, M. Hide¹

¹Department of Dermatology,

²Department of Cardiovascular Internal Medicine,

³Department of Orthopedic Surgery,

⁴Department of Cardiovascular Surgery, Hiroshima University,

⁵Department of Dermatology, Mazda Hospital, Hiroshima, Japan

Introduction: Diabetic foot ulcer occurs from various kinds of factors including blood flow, metabolism, infection, pressure and so on. Among these factors, the assessment of the blood flow is an essential and key step in the treatment of the ulcer. In this study, we measured skin perfusion pressure (SPP) of patients with diabetic foot ulcer and analyzed the correlation between ulcer healing and the value of SPP. A logistic regression analysis predicted the probability of ulcer healing by using a SPP value.

Abstracts

Methods: We assessed the SPP of a total of 64 toes with refractory foot ulceration in 38 patients with diabetic mellitus.

Results: In most of the healed toes, the SPP values were higher than 30 mmHg. However, in patients with poor-controlled glycemia and severe infectious gangrene, their toe ulcers were not healed in spite of high values of SPP much more than 30 mmHg. According to a logistic regression analysis, foot ulcer healing probability was calculated as 95% when the SPP value was 50 mmHg and 33% when the value was 30 mmHg.

Conclusion: In the treatment of diabetic foot ulcer, we should take account of infection control metabolism control and pressure reduction if blood flow is good. The SPP measurement is a useful modality to estimate peripheral blood flow and to determine the suitable treatment of diabetic foot ulcer.

S6-11

Clinical comparison study of foot ulcers among diabetic mellitus, spina bifida, and Hansen's disease

Terashi H., Tsuji Y., Kitano I., Ohira Y., Yamaguchi A., Nogami R., and Tahara S

Departments of Plastic Surgery, Kobe University, and Wound therapy center, Shin-Suma Hospital, Kobe, and Kikuchi Keifuen National Hospital, Kumamoto, Japan

Background: Those who are ambulatory with sensory disturbance have always the risk to make wounds on their own foot.

Aim: The aim of this study is to compare three disorders clinically which are diabetic mellitus, spina bifida, and Hansen's disease.

Methods: We have experienced the patients with various foot ulcerations including a few hundreds of diabetic mellitus, ten of spina bifida, and seven of Hansen's disease. We compared the characteristics of each foot ulcer among three disorders clinically.

Results: The number of the patients of diabetic mellitus is recently soaring up and they are apt to have also PAD. So, the multi-disciplinary approach is necessary for the proper therapy. Spina bifida is a congenital disorder. So, it is need for the patients to recognize the risk of making wounds easily. Hansen's disease has also physically challenged hands and so it is difficult to care their feet by their own hands.

Conclusions: They have common need of their own tailor-made footwear for each foot.

JAPAN KOREAN SYMPOSIUM: UPDATE STEM CELL BASED REGENERATION MEDICINE JK-02

Adipose-derived stem cells for wound repair and regeneration

Hiroshi Mizuno, MD

Department of Plastic and Reconstructive Surgery, Nippon Medical School, Tokyo Japan

Adipose-derived stem cells (ASCs) are promising for future cell-based therapy such as regenerative medicine since adipose tissue is plentiful, easily obtainable with less donor site morbidity and patient discomfort compared with bone marrow procurement. Our laboratory has investigated the versatility of ASCs for tissue regeneration and shown that ASCs have the capability of differentiating into a variety of cell lineages both *in vitro* and *in vivo*.

In addition to tissue regeneration, topical administration of bone marrow-derived stem cells in skin wound has been shown to contribute for accelerating wound healing with less scarring, indicating that mesenchymal stem cells might be expected for wound healing as well as regenerative medicine. Our preliminary data have also suggested that ASCs might accelerate the primary wound healing in several tissues *in vivo*.

This paper highlights our research work about ASCs and future clinical application in the field of tissue regeneration and wound healing.