Collagen I matrices containing high-sulfated HA modulate phenotype and function of human pro-inflammatory M1 macrophages  
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In chronic wounds sequence of normal wound healing progressing through inflammation, granulation tissue formation and remodelling often fails due to prolongation of the inflammatory phase. Unsuppressed activation of inflammatory macrophages (M1) is assumed as major cause for persistent inflammation. Thus biomaterials capable to modulate macrophage activation and to promote inflammatory resolution represent a promising approach for treatment of non healing wounds. Since native ECM is known to guide functions of immune cells we tested artificially compound ECM (aECM) on their capability to modulate the function of inflammatory M1. Artificial ECM were composed of collagen I and the glycosaminoglycan hyaluronan (HA) which was additionally modified by introduction of sulfate groups resulting in low- and high-sulfated HA derivatives. Testing functions of M1 revealed reduced inflammatory activities of macrophages differentiated on matrices composed of collagen I and high-sulfated HA (hsHA) as seen e.g. by reduced release of TNFα and IL-12 due to impaired activation of NFκB. Moreover, these macrophages are capable to secrete immuno-regulatory IL-10 typically not produced by M1. Cytokine ratio of IL-12/IL-10 created by macrophages on this aECM is similar to that produced by regulatory M2 macrophages. Since these macrophages also show reduced activity of transcription factors STAT and IRF both controlling macrophage polarization to M2 subsets we conclude that collagen I matrices containing hsHA dampen inflammatory macrophage function by impeding signaling pathways crucial for polarization and activation of pro-inflammatory M1. We therefore suggest this aECM as promising biomaterial for modulating inflammatory macrophage functions during wound healing.

Phychochemical incorporated biomaterials for wound healing  
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efficacy of natural compound encapsulated biomaterials in wound care management is gaining attention now a days. Systems capable of delivering bioactive agents into cutaneous/subcutaneous levels are of great interest to therapeutic or cosmetic approaches for effective treatment and acceleration of skin wound healing. C-phycocyanin, a chromo-protein from cyanobacteria was impregnated/dispersed cross-linked with PLL-PLA (GA) into collagen solution to form biofilms, bioscaffolds and bio-based cryogels by polymerization, interlinking and cryogelation techniques respectively. Tensile strength, swelling test, drug release rate, porosity and in vitro biodegradation rate were evaluated. In vitro and in vivo models of wound healing were carried out by standard methods. Rate of fibroblast migration and proliferation was calculated during the treatment period. In vivo studies on human dermal fibroblasts proved that C-phycocyanin bioscaffolds could retain the cellular cyto-compatibility and effectively accelerate cell migration and proliferation through cyclin dependent kinase pathways and changes in cell cycle patterns. In vivo animal test further revealed that scab-off could sufficiently support and accelerate the fibroblast infiltration from surrounding tissue to wounded area and also accelerate wound contraction. Among all biomaterials tested, cryogel proved to be better in terms of tensile-strength and hydro-absorption rate. The collagen-1 molecules in cryogel scaffold cross-linked with PLL(GA) is a potential candidate for dermal equivalent with enhanced bio-stability and cryogels have higher bio-compatibility with faster biodegradability. The suitability of cryogel was also observed in fibroblast-keratinocytes co-culture condition which closely mimics the artificial skin.

Human dermis-derived ABCB5-positive mesenchymal stem cells accelerate mouse skin full-thickness excisional wound healing in part by the secretion of interleukin-1 receptor antagonist  
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Mesenchymal stem cells (MSCs) feature many characteristics, such as tissue regeneration capacity and immune modulation, beneficial for therapeutic applications in injury and trauma. With respect to wound healing, MSCs have been proposed to suppress inflammatory processes and stimulate repair mechanisms such as myofibroblast differentiation and matrix deposition, angiogenesis, as well as re-epithelialisation. Although MSCs are present in all connective tissues of the body including the dermis, these studies have mainly focused on MSCs isolated from either bone-marrow or adipose tissue. Here we describe the isolation of an ATP-binding cassette sub-family B member 5 (ABCB5) positive plastic-adherent dermal cell subpopulation and its characterization as bona-fide MSCs. ABCB5+ dermal MSCs contributed to full-thickness excisional skin wound healing in mice to a comparable level as GAP isolated and cultured bone-marrow derived MSCs. Furthermore, we demonstrated that ABCB5+ dermal MSCs secreted interleukin-1 receptor antagonist (IL-1RA) in response to inflammatory stimulation, which in turn inhibited classical macrophage activation with TNF-alpha release. IL-1RA inhibits the action of IL-1 cytokines by binding to the IL-1 receptors without activating signal transduction. The importance of MSC-secreted IL-1RA for the observed anti-inflammatory activity was substantiated by the absence of spontaneous cutaneous wound healing in mice was substantiated by a uPAR-mediated gene-silencing approach. In conclusion, human dermal ABCB5+ sorted MSCs appear as an essential source for cell-based therapy of skin wounds that accelerates healing at least in part by the secretion of IL-1RA.
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Rattle oils promote keratinocyte cell growth and inhibit leukocyte activation

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Emu oil has been used as a popular traditional wound healing treatment and anti-inflammatory remedy by Australian aborigines. Several published animal studies suggest emu oil treatment can promote reepithelialization in wound areas and may have anti-inflammatory properties. We investigated effects of 6 different individual emu oils along with oils from other rata family (3 ostriches with 1 rhea, ducks, tea tree and olive oil) on immortalized human keratinocytes (HaCaT cells) in vitro with 0%, 0.5% and 1.0% concentrations in culture medium. Human peripheral blood mononuclear cells (PBMC) were subjected to phytohemagglutinin (PHA) activation and exposed to each oil at 0.5% concentration in culture medium for 48 hours incubation to evaluate its impact on PBMC’s survival rate and NF-κB production with ELISPOT Assays. Shorter population doubling time durations were observed for PBMC cells cultured in emu oil culture media (0.5% and 1.0% emu oil) compared to PBMC cultured in PBMC media only. Inhibition of NF-κB signaling by hypochlorite rejuvenates skin. We found that emu oil from different species had significantly different impacts on PHA-activated PBMCs. Emu oil from the same species had concentration dependent effects. Interestingly, emu oil from the same species had concentration and species dependent effects on PHA-activated PBMCs. This shows that emu oil treatment may have potential to increase wound healing in humans by impelling growth rate of keratinocytes and confirms its anti-inflammatory properties on PBMC. With this combination, rattle oils may be useful as a component in treatments for wound healing and inflammatory skin conditions.

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Inhibition of NF-κB signaling by hypochlorite rejuvenates skin

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Hypochlorite, the active ingredient in Clorox, is used worldwide for cleaning. As a chemical treatment, 0.005% hypochlorite bath is the same concentration used in swimming pools; improves atopic dermatitis, which may be mediated by antimicrobial and/or anti-inflammatory effects. We’ve discovered that hypochlorite inhibits signaling governed by nuclear factor (NF-κB), thereby inhibiting inflammation and reepithelializing agents that in skin. In cultured cells, hypochlorite exposure leads to oxidation of regulatory cysteines and inactivation of IkκB kinase (IKK), a key regulator of NF-κB activation. In aged mice, topical hypochlorite reduced skin NF-κB signaling in vivo, and attenuated age-dependent expression of p16(INK4a) and other genes, leading to a striking re-acquisition of juvenile skin phenotypes. These included enhanced epidermal thickness and proliferation, which reverted to pre-inflammatory keratinocyte withdrawal. Our work defines a new mechanism for a commonly used dermatologic treatment. Discovery of chemical methods that reversibly modulate IKK signaling should advance our ability to control diverse physiological and pathologic skin processes regulated by the NF-κB pathway.

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Impaired β2 integrin-dependent activation of NOX2 in macrophages delays wound healing in CD18−/− mice

A. Küchler1, S. Schacht1, S. Vander Beke1, B. DeClercq1,1, C. Hausser1, A. Rück1, P. Hawkins1,1,1,2,3, K. Schaffler-Kochanek1, and a Sindl1, 1 Department of Dermatology and Allergie Diseases, University of Ulm, Ulm, Germany, 2 University of Ghent, Ghent, Belgium, 3 University of Leuven, Leuven, Belgium, and 1 Leukocyte Adhesion Deficiency Type1 (LAD1) patients with reduced levels of β2 integrins due to mutations in their common β chain (CD18) suffer from severe wound healing defects. In the CD18−/− murine LAD1 model we previously found β2 integrins to control phagocytosis of apoptotic neutrophils (PMN) by macrophages (MΦ) with subsequent release of reactive oxygen species (ROS) and active TGF-β (as wound sites and thus normal wound healing). The NADPH oxidase NOX2 is the main source of ROS release in MΦ. We here investigated whether impaired phagocytic activation of NOX2 in MΦ is causal for insufficient ROS release, reduced TGF-β function and impaired wound healing in CD18−/− mice. Using a variety of in vivo imaging and wound healing experiments we showed that CD18−/− mice wounds mount significantly reduced amounts of ROS compared to wildtype (WT) wounds. Further, the wound healing defect of CD18−/− mice was reversed by injection of the oxidase burst inducer Rutinose. Interestingly, significantly increased active TGF-β release at wound sites and NOX2 activation by MΦ upon phagocytosis of apoptotic PMN in vitro. NADPH based fluorescence lifetime imaging revealed NOX2 to be activated in WT but not in CD18−/− MΦ upon adhesion to apoptotic PMN. Similar to CD18−/− mice NOX2-deficient p67phox-/- mice presented significantly delayed wound healing compared to WT mice. Importantly, injection of WT but not p67phox-/- MΦ around CD18−/− wounds fully restored the LAD1 wound healing defect. Taken together, β2 integrin-dependent NOX2 activation is essential for sufficient ROS production with subsequent TGF-β function and MΦ normalization healing. Targeting β2 integrins in β2 integrins deficient mice could be of therapeutic interest in LAD1 and other disorders.

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Fractional laser-assisted changing of tissue shape

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There is a strong need to change the shape and to predictably move soft tissue in a particular direction, for example to relieve tension on burn scars, close a wound or treat skin conditions. Conventional fractional ablative laser typically removes 10-30% of the skin volume by ablating very small channels deeply into skin. These microscopic holes “fill in” by fibroblast migration into the channels and new tissue is formed. Instead of letting the holes “fill in”, we tested the hypothesis that closing them (similar to macroscopic wound closure) would reduce the skin area, and move skin in the direction of hole closure. Ex vivo human skin samples were treated with a prototype fractional CO2 laser device operating at 10 Hz, 100 mJ pulse, 0.1 mm spot, 1000 Hz exposure rate to produce arrays of holes of 2 mm depth. We found that the residual thermal damage layer (RTD) surrounding each hole, typical of fractional lasers, was still and prevented hole closure. A contact Zefi window apparatus was designed and used to entrap laser-induced steam inside the skin tissue, breaking up the RTD. This produced a significant increase in hole eccentricity (p=0.00 and circumference (p=0.027), with fractioning of the RTD seen by histology which allowed easier hole closure. We then stretched and applied a nanogel, elastic surgical dressing to provide hole closure by elastic recoil of the dressing. In an in vivo swine study, epilated abdominal test sites were marked with sterile ink micro tattoos prior to fractional laser treatments with and without (control) hole closure. At 7, 14 and 28 days there was significant reduction in skin area due to hole closure, specifically in the direction of hole closure. There was no scoring. In conclusion, we show that a modified fractional ablative laser treatment followed by closure of microscopic holes, can reduce skin area and move skin in a preferred direction. This approach appears promising for clinical applications.

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Interest of Rhabdöbabt© elphant tranquilization on epidermal repairation

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Epidermal repairation is a crucial step in cutaneous restoration of intimal skin and in wound healing. It involves the combination of two cellular processes, proliferation and migration, temporally and locally orchestrated by specific molecular mediators: growth factors, cytokines, collagen, hyaluronic acid, etc. The restoration of barrier function is also important for epidermal reparation. Ectopic expression of Runx2 possibly plays a role in the hyper-responsiveness of keloid to mechanical stimulation. By using atomic force microscopy, we found that KFs (1,205 ± 145 pascal) (N=7, P=0.0744), while keloid tissue and normal skin tissue were measured at 16,570 ± 1400 pascal (N=5, P=0.005). We then performed a study to understand the mechanical properties of KFs and the role of nuclear Runx2, an osteogenic and chondrogenic transcription factor, in their responsiveness to mechanical stimulations. KFs and NFs display different profile of biomechanical properties and response to changes in substratum stiffness. Ectopic expression of Runx2 possibly plays a role in the hyper-responsiveness of keloid fibroblasts to mechanical stimulation. The purpose of the study is to understand the mechanical properties of KFs and the role of nuclear Runx2, an osteogenic and chondrogenic transcription factor, in their responsiveness to mechanical stimulation. By using atomic force microscopy, we found that KFs (1,205 ± 145 pascal) (N=7, P=0.0744), while keloid tissue and normal skin tissue were measured at 16,570 ± 1400 pascal (N=5, P=0.005). We then performed a study to understand the mechanical properties of KFs and the role of nuclear Runx2, an osteogenic and chondrogenic transcription factor, in their responsiveness to mechanical stimulations. KFs and NFs display different profile of biomechanical properties and response to changes in substratum stiffness. Ectopic expression of Runx2 possibly plays a role in the hyper-responsiveness of keloid to mechanical stimulation. In conclusion, KFs and NFs display different profile of biomechanical properties and response to changes in substratum stiffness. Ectopic expression of Runx2 possibly plays a role in the hyper-responsiveness of keloid to mechanical stimulation.
High concentration of glucose activates migration and proliferation of human skin keratinocytes through inducing active release of HMGB1

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High-mobility group box 1 (HMGB1) is a nuclear factor and a secreted protein. During inflammation, HMGB1 is secreted into the extracellular space where it can interact with the receptor for advanced glycation end products (RAGE) and trigger proinflammatory signals. This protein has also been shown to function as a cytokine and to promote keratinocyte scratch wound healing. In the present study, we investigated the effect of a high glucose concentration on secretion of HMGB1 in cultured human skin keratinocytes. A high concentration (10 mM) of glucose decreased the high concentration of glucose induced HMGB1 release in human skin keratinocytes and promoted phospho- p38 expression (by 30-fold, p<.01). Moreover, the expression of HMGB1 in keratinocytes was increased 10-fold by excess glucose. SNA-induced GM3S depletion increased glucose transporter 1 (GLUT1) translocation to the cell membrane (by 2 fold, p<.001), and migration (p<.01) compared with untreated and scrambled SNA-treated controls. These results suggest that a high glucose concentration induces HMGB1 release from skin keratinocytes and may enhance wound healing in the skin.

Cytoplasmic sequestration of keratinocyte GLUT1 by ganglioside GM3 mediates impaired vessel integrity

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Recent publications featured the lymphatic function on adipose tissue research including skin. In mice, a naturally occurring mouse model of lymphedema due to hereditary inactivating mutations in VEGFR 3, exhibit adipose layer accumulation in skin. In humans, excess adipose tissue is frequently found in lymphedema patients, suggesting that the impairment of lymphatic function resulted in adipose tissue accumulation. We have recently shown that apelin/APN signaling promotes the migration and proliferation of blood and lymphatic vessels, resulting in attenuation of UV-induced inflammation. Here we first describe a link between adipocytes and the integrity of blood/lymphatic endothelial cells in skin. Apelin-deficiency resulted in increased subcutaneous layer of adipocytes, whereas apelin overexpression inhibited its accumulation by enhancing vessel integrity. The co-cultures of lymphatic endothelial cells and adipocytes revealed that adipocyte differentiation was potently induced by the plasma from a high-fat diet through the endothelial monolayer, whereas no significant difference was found in the absence or presence of plasma from a regular diet. Moreover, oleic acid, one of fatty acids rich in a high-fat diet induced hyperpermeability of endothelial cells with loss of VE-cadherin localization on cell-cell junctions, causing adipocyte differentiation, whereas apelin inhibited vascular hyperpermeability by promoting vascular stabilization. These results indicate that apelin inhibits high-fat-diet-induced obesity and subcutaneous adipose tissue accumulation by enhancing vessel integrity. Apelin could serve as a therapeutic target for treating adipose tissue accumulation and its related diseases.

Apelin inhibits subcutaneous adipose tissue accumulation by enhancing lymphatic and blood vessel integrity

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Adipose tissue accumulation frequently occurs in obesity and its related diseases. Here we provide the evidence that apelin is an adipose tissue-resident hormone that inhibits adipose tissue accumulation by enhancing lymphatic and blood vessel integrity. The co-cultures of lymphatic endothelial cells and adipocytes revealed that adipocyte differentiation was potently induced by the plasma from a high-fat diet through the endothelial monolayer, whereas no significant difference was found in the absence or presence of plasma from a regular diet. Moreover, oleic acid, one of fatty acids rich in a high-fat diet induced hyperpermeability of endothelial cells with loss of VE-cadherin localization on cell-cell junctions, causing adipocyte differentiation, whereas apelin inhibited vascular hyperpermeability by promoting vascular stabilization. These results indicate that apelin inhibits high-fat-diet-induced obesity and subcutaneous adipose tissue accumulation by enhancing vessel integrity. Apelin could serve as a therapeutic target for treating adipose tissue accumulation and its related diseases.

Vascular hyperpermeability and severe impairment of lymphatic vessels contribute to the formation of pressure ulcers in mouse ichimia-reperfusion injury

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Ichimia-reperfusion (IR) injury plays a major role in developing pressure ulcers. However, it remains unclear how the IR injury affects the structure and function of blood or lymphatic vessels and how these affects contribute to the formation of pressure ulcers. To address the question, we investigated the biological impact of IR injury on cutaneous vasculature in a mouse IR injury model utilizing ceramic magnets. We initially subjected wild-type mice to the standard regimen, and found that cutaneous IR injury develops marked edema and subsequent ulceration. Therefore, we next quantitated the vascular leakage in vivo by intravenous injection of fluorescence-labeled polyion complex nanoparticles which are 100nm in diameter. Two hours after IR, marked increase of fluorescence was found in the areas of IR injury, indicating that vascular hyperpermeability was induced in the experimental cutaneous IR injury. Second, we quantitated the lymphatic drainage in vivo using indocyanine green (ICG). Intratumoral injection of ICG indicated that adjacent area of IR injury revealed that the lymphatic transport of ICG was severely impaired in the areas of IR injury 24 hours after IR, whereas sham-treated skin showed the complete transport of ICG by normal lymphatic vessels. Double immunofluorescence stains for blood and lymphatic vessels revealed that lymphatic vessels were markedly enlarged, whereas blood vessels were found in the areas of IR injury, indicating that the lymphatic vessels are totally abolished. These results demonstrate that the increased leakage from blood vessels and the lack of lymphatic drainage contribute to the formation of cutaneous edema and ulceration in the mouse IR injury model.
1467 Smad7 accelerated healing of cutaneous wounds associated with chronic inflammation

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We have previously shown that overexpression of the TGFβ1 antagonist Smad7 in mouse epidermis accelerates skin wound healing. In this study, we found increased TGFβ1 expression and inflammatory cell infiltration in chronic ulcers of diabetic patients. To assess if Smad7 has the potential to treat diabetic wound healing associated with chronic inflammation, we bred K5.Smad7 transgenic mice with K5.TGFβ1 mice, the latter exhibits a chronic inflammatory skin phenotype and delayed healing of excisional wounds. We found that bigenic K5.TGFβ1/Smad7 mice had reduced inflammation and delayed wound healing. Next, we produced recombinant human Smad7 protein with a Tat-tag (Tat-Smad7) that rapidly permeates the cell membrane and enters the nucleus. In vitro tests show that Tat-Smad7 rapidly entered keratinocytes in culture and remained in cells for at least 16 hours. Tat-Smad7 treatment was capable of blocking TGFβ1-induced phosphorylation of Smad2 in keratinocytes. Subcutaneous injection of Tat-Smad7 into the skin of K5.TGFβ1 mice significantly alleviated skin inflammation. When Tat-Smad7 protein was topically applied to 6-mm wounds on K5.TGFβ1 transgenic mice, it accelerated healing by promoting re-epithelialization and reducing inflammation. Molecular analyses revealed that Smad7 directly bound to the promoter of Rac1 and transcriptionally upregulated Rac1 expression, resulting in accelerated keratinocyte migration. In addition, Smad7 treated wounds have shown reduced staining of pSmad2 and NFKB p65, suggesting that Smad7 blocking of both TGFβ1 and NFKB signaling pathways is sufficient to overcome chronic inflammation, thus improving the wound microenvironment for healing. Our study suggests that Tat-Smad7 could be developed into a novel therapeutic agent to treat chronic inflammation-associated wounds by accelerating re-epithelialization and inhibiting inflammation.

1468 IFC-CAF, extract from Crotophorus aspera's eggs significantly promotes skin homeostasis, migration and survival of skin cells in vitro

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ICF-CAF, an extract derived from Crotophorus aspera's eggs, has been traditionally used in medical preparations due to its anti-inflammatory and anti-pigmentation properties. It is known to contain high levels of thiosulfate and sulfur compounds that are reported to inhibit proliferation and migration of keratinocytes. This study aimed to evaluate the potential of IFC-CAF in promoting skin homeostasis by inducing keratinocyte migration. Fibroblasts and keratinocytes were cultured and treated with IFC-CAF. The migratory activity of keratinocytes was determined using a wound healing assay. The results showed a significant increase in the migratory activity of keratinocytes treated with IFC-CAF compared to control groups. Additionally, Western blot analyses revealed an increase in the expression of epithelial-mesenchymal transition (EMT) markers such as N-cadherin and Snail, which are known to promote cell migration. These findings suggest that IFC-CAF has potential therapeutic applications in skin wound healing and tissue regeneration.

1469 Development of a clinical grade suspension of allogeneic human dermal fibroblasts with extended shelf life capable of intradermal delivery through small caliber needles

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Injectable autologous fibroblast preparations have a history of use and are approved in the US for the treatment of nasal deformities. We report on the development and characterization of an allogeneic dermal fibroblast cell bank and the development of an injectable cell suspension with an extended shelf life for intradermal delivery. The cell suspension was prepared by trypsinization and centrifugation of allogeneic dermal fibroblasts in a series of clinical studies in the UK and US including a study for the treatment of Epidermolysis Bullosa. This material can be shipped internationally and has been used in a series of clinical studies in the UK and US including a study for the treatment of Epidermolysis Bullosa that is presented at this meeting. This presentation will outline the GMP and US/UK regulatory requirements for producing such cell suspensions.

1470 Engineered wound-dressing carriers with peripheral blood-derived angiogenic factor protein mixtures: Therapeutic implications for peripheral ischaemic tissue

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Engineered wound-dressing carriers with peripheral blood-derived angiogenic factor protein mixtures have been developed as a potential therapy for peripheral ischaemic tissue. We compared the efficacy of three different carrier materials: collagen gel, fibrin gel, and collagen/fibrin gel, in promoting angiogenesis in vitro and in vivo. The carriers were loaded with hypoxia-induced angiogenic factor mixtures (HIF-1α, VEGF, and TGFβ) and applied to a murine dermal wound model. The results showed a significant increase in blood vessel density and neovascularization in wounds treated with the HIF-1α, VEGF, and TGFβ carrier, compared to controls. These findings suggest that engineered wound-dressing carriers with peripheral blood-derived angiogenic factor protein mixtures have therapeutic potential for the treatment of peripheral ischaemic tissue.

1471 Matrix metalloproteinases 1, 2, 13 and 14 are differentially expressed in keloid scars compared to normal skin

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Keloids are fibroproliferative scars that form in response to abnormal healing processes. There is evidence that abnormal matrix metalloproteinase (MMP) expression may contribute to keloid formation. In this study, we aimed to investigate the expression of MMPs 1, 2, 13, and 14 in keloid scars compared to normal skin. Immunohistochemistry and western blot analyses were performed on keloid and normal skin samples. The results showed that MMP1, MMP2, MMP13, and MMP14 were differentially expressed in keloid scars compared to normal skin. MMP1 and MMP2 were upregulated in keloid scars, while MMP13 and MMP14 were downregulated compared to normal skin. These findings suggest that altered MMP expression may play a role in the pathogenesis of keloids.

1472 Mast cells are dispensable for granulation tissue formation during skin wound healing and do not impact tissue fibrosis in mice

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Impaired wound healing, defective regeneration as well as fibrosis of diverse tissues are leading causes of morbidity and mortality. The role of mast cells in tissue repair and regeneration is still lacking. This study aimed to investigate the role of mast cells in skin wound healing and fibrosis formation. Mast cells were depleted from mice using an anti-CD11b antibody. The results showed that mast cells were not essential for skin wound healing, as the healing process was comparable in mast cell-depleted and control mice. However, mast cell depletion significantly reduced scar formation, as assessed by collagen deposition and fibrosis markers. These findings suggest that mast cells are dispensable for granulation tissue formation during skin wound healing and do not impact tissue fibrosis in mice.
Angiopoietin-like 4 improves diabetic wound healing

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Asai et al. demonstrated that pericytes are major sources of the secreted glycoprotein and integrin-ligand MFG-E8 in B16 melanoma tumors, and that MFG-E8 promotes angiogenesis via enhanced interaction of PDGF (PDGF) receptor β (PDGFR-β) signaling mediated by integrin-growth factor receptor cross-talk. However, functions of MFG-E8 in angiogenesis in cutaneous wound healing are not well characterized. The objective of this study was to ascertain the role of MFG-E8 in cutaneous wound healing. First, we examined the distribution of MFG-E8 in normal murine and human skin in vivo. In the dermis, accumulations of MFG-E8 were found around CD31+ blood vessels, and MFG-E8 co-localized with the pericyte marker. Next, we examined the role of MFG-E8 in a cutaneous wound healing mice model. Protein and mRNA levels of MFG-E8 expression in the dermis was elevated during full-thickness wound healing by immunofluorescence staining and real-time PCR. In addition, MFG-E8 localization differed in granulation tissue, as well as around blood vessels in the dermis. Wound healing was delayed in MFG-E8 KO mice compared with WT mice, and vessel numbers in wound areas were reduced in MFG-E8 KO mice. Inhibition of MFG-E8 production by siRNA attenuated the formation of capillary-like structures in diabetic wounds. These results indicate that the epidermal expression of podoplanin in cutaneous wound healing is inhibited by a negative regulatory role of platelets.

Recipe for impaired wound healing: Cross-talk between adrenergic and toll-like receptors in mesenchymal stem cells and keratinocytes

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We recently demonstrated that pericytes are major sources of the secreted glycoprotein and integrin-ligand MFG-E8 in B16 melanoma tumors, and that MFG-E8 promotes angiogenesis via enhanced interaction of PDGF (PDGF) receptor β (PDGFR-β) signaling mediated by integrin-growth factor receptor cross-talk. However, functions of MFG-E8 in angiogenesis in cutaneous wound healing are not well characterized. The objective of this study was to ascertain the role of MFG-E8 in cutaneous wound healing. First, we examined the distribution of MFG-E8 in normal murine and human skin in vivo. In the dermis, accumulations of MFG-E8 were found around CD31+ blood vessels, and MFG-E8 co-localized with the pericyte marker. Next, we examined the role of MFG-E8 in a cutaneous wound healing mice model. Protein and mRNA levels of MFG-E8 expression in the dermis was elevated during full-thickness wound healing by immunofluorescence staining and real-time PCR. In addition, MFG-E8 localization differed in granulation tissue, as well as around blood vessels in the dermis. Wound healing was delayed in MFG-E8 KO mice compared with WT mice, and vessel numbers in wound areas were reduced in MFG-E8 KO mice. Inhibition of MFG-E8 production by siRNA attenuated the formation of capillary-like structures in diabetic wounds. These results indicate that the epidermal expression of podoplanin in cutaneous wound healing is inhibited by a negative regulatory role of platelets.

Podoplanin enhances migration of keratinocytes by down-regulation of E-cadherin

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C Reactive protein (CRP) is an acute phase protein that is closely related to age-related inflammatory and degenerative diseases. The role of CRP in cardiac regeneration is currently controversial. In this study, we evaluated the effects of CRP on wound healing and cardiac regeneration and transplanted cells. Furthermore, we investigated the role of CRP in cardiac regeneration in a mouse model. We found that CRP inhibited wound healing and cardiac regeneration. In addition, we demonstrated that CRP inhibited the proliferation of hESC-CM and hESC-CM-CXCL12. These findings suggest that CRP may play a role in cardiac regeneration.

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1479 A novel, simplified, non-cultured autologous cellular grafting procedure for chronic non-healing ulcers
AC Stegeman, KB Goh and BY Tang National Skin Centre, Singapore, Singapore
Skin substitutes have been used as adjuvant therapy in chronic non-healing wounds. We investi-
gated a novel, simplified non-cultured, autologous cellular grafting procedure using a ‘wet-plate’ tech-
tique to treat chronic ulcerating wounds. This was a prospective pilot study that involved har-
custing a ultra-thin split skin graft from the patient’s gluteal region which was washed, separated
and prepared in 6 different wells to obtain an autologous mixture of keratinocytes, melanocytes and
fibroblasts that was subsequently applied directly to the wound via a hyaluronic acid matrix. There
were 9 patients with chronic ulcerating ulcers of different etiology recruited between April 2008 to May 2011 at the National Skin Centre, Singapore. A total of 16 ulcers were analysed with an average size of 47 cm² (range 0.5–149.5, SD 39.6) and mean duration of 3 months (range 3 days–68.5, median 12). Outcome evaluation was performed via digital photography, direct
tracing and quality of life assessment using the Cardiff Wound Impact Schedule (CWIS). At 3 months
after the 1st grafting, the mean re-epithelialisation was 57.9% (range 6.8–100, SD 35) with com-
plete healing in 2 pts, >50% re-epithelialisation in 3 pts, 25–50% re-epithelialisation in 2 pts and
<25% re-epithelialisation in 2 pts. 4 and 2 pts had repeat 2nd and 3rd grafting procedures done respec-
tively at 4 weeks intervals. At 3 months after the final graft, 4 pts had further re-epitheliali-
sation (range 59.9–79.9). A follow up period of 6 months demonstrated 100% healing of wounds.
These results suggest that this simplified non-cultured autologous cellular

grafting technique may be beneficial for treating chronic ulcers that have failed standard therapy. Dis-
tinct advantages of this technique include ease of use in clinical practice, economical, short
preparation time and the ability to treat large wounds in a single procedure.

1481 Audible doppler signal versus ankle brachial pressure index in patients with leg ulcer
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Peripheral vascular disease (PAD) of lower extremity affects 12% of older general population. The
vascular assessment is required for all leg ulcers to confirm a good vascular flow for compression
therapy. The non-invasive vascular assessment tools include Ankle Brachial Pressure Index (ABPI),
toe pressure and full arterial segmental leg Doppler. About 80% of people with diabetes have non-
compressible arterial vessels and 20% of non-diabetics also have calcified vessels. These individu-
als have unreliable ABPI. There is a need for an alternative quick bedside assessment method at
point of care to support clinical findings. Methodology We conducted a study on 200 consecutive
patients referred to wound care clinic. The results from a full segmental leg handheld Doppler (HD-
LDI) compared to the results from audible signals (monophasic, biphasic, triphasic) done by
hand held doppler at bedside. Results: Triphasic and biphasic HDLD signals indicate ABPI of 0.9 or
greater. Presence of biphasic signals indicate ABPI of 0.9 or greater in a group of patients with
infarct foot ulcer only monophasic signals indicate ABPI of less than 0.9. Audible doppler has high
sensitivity and submucosal specificity in detection of significant PAD if persons with diabetes are
included. LDLI can be used with limited ABPI <60%. S0.6212 Conclusion. Audible Doppler signal
waves have a high degree of accuracy for the detection or exclusion of PAD compared with the ABPI using
the cut off point of 0.9 or lower. The effectiveness of HDLD signal detection should be encour-
aged through a physical examination along with audible doppler signals.

1483 Novel synthesis and activity of solenopsin A and analogs, topical inhibitors of phosphoins-
ositide 3-kinase/akt with ceramide-like properties
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Solenopsin A is a small molecule that is the principle alkaloid in fire ant venom (Solenopsis invicta),
we have previously demonstrated that solenopsin A is an inhibitor of phosphoinositide-3 kinase/Akt
signaling and angiogenesis (Blood 2007) and of Pseudomonas quorum signaling (J Infect Dis 2008).
Based on the structural similarity of solenopsin A to ceramide, a lipid signaling molecule that also
inhibits Akt, we performed live-cell imaging experiments to monitor PI3K/Akt signaling in various
3T3 cells. We have previously demonstrated that solenopsin A inhibits phosphoinositide-3 kinase/Akt
signaling and angiogenesis (Blood 2007) and of Pseudomonas quorum signaling (J Infect Dis 2008).
We investigated the effects of solenopsin A on cell proliferation in various human cell lines.
Solenopsin A and analogs were successfully obtained after paldidomide-catalyzed hydrogenation of
the various 2,3-dihydropyridines. The synthetic solenopsin and novel analogs was also shown to
determine constitutive Akt activity in A549 adenocarcinoma cells. We have thus made solenopsin A and analogs amenable to large scale synthesis. Topical solenopsin and analogs have potential for treatment of cutaneous neoplasms, through mechanisms
distinct from inhaled and 5-fluorouracil.

1484 Collagen XVII ectodomain shedding alters keratinocyte proliferation and morphology through
modulation of cell adhesiveness
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Dermatology, University of Oslo, Oslo, Finland
The hemi-segmental colagen deficiencies are crucial for the anchorage of the epidermis to the
basement membrane. We have previously demonstrated that the collagenous ectodomain of this
large collagen protein is constitutively shed from the cell surface by disintegrins-metalloproteinases
(ADAMs). The physiological function of collagen XVII shedding is still unclear. To investigate this
we generated transgenic collagen XVII non-shedding mice (Col17A1) by introducing a deletion
of 41 amino acids within the linker domain that contains the sheddase cleavage site. Exclusive
expression of this mutant did not interfere with early skin architecture, epidermal differentiation
or formation of the basement membrane. To verify previously observed in vitro alterations in cell
morphology in conditioned acute skin wounds were induced in the Col17A1 mice. Wound closure was sig-
nificantly accelerated in Col17A1 mice, with increased epithelial tongues and enhanced ker-
atinocyte proliferation, especially during early re-epithelialization. This cell autonomously driven
production of collagen XVII and of Pseudomonas quorum signaling (J Infect Dis 2008).

1480 Development of 3D scaffolds for the investigation of chronic wounds
K Karlsson, R Steen, J Whitting, L Smith, D Israel, F Bamgboye, R Dawson, T Dargaville, A Cosmin, D Adams and D Lang
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to investi-
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of 41 amino acids within the linker domain that contains the sheddase cleavage site. Exclusive
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atinocyte proliferation, especially during early re-epithelialization. This cell autonomously driven
production of collagen XVII and of Pseudomonas quorum signaling (J Infect Dis 2008).

1482 MiR-29b as a potential therapeutic target of radiation-induced skin fibrosis
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WI
Excessive collagen deposition resulting in skin fibrosis is a leading cause of skin dysfunction in the
late stages of radiation-induced skin injury. MicroRNAs (miRNAs) are epigenetic regulators of gene
expression and have been implicated in regulation of cellular responses to ionizing radiation. The
aim of the present study was to identify differentially expressed miRNAs in irradiated rat skin using
high-throughput Illumina sequencing technology and to identify their post-transcriptional
functions. Adult male rats were irradiated (n=6) with a single dose of 30 Gy to the skin, sham-irradiated rats
(n=6) served as a control. Irradiation to the skin was limited to the full thickness of the skin only. At
48 hours or 30 days after irradiation, rats were euthanized and skin miRNA fractions were used to
generate cDNA libraries which were amplified through polymerase chain reaction (PCR). Deep sequencing of miRNAs in skin resulted in approximately 1.5 gigabytes of data from each exper-
imental sample. TruSeq detected on average 26k known mature miRNAs per sample (ranging 184 to
224) and 192 novel miRNAs per sample (176 to 244). miR-29b was identified among the top dif-
erential expressed miRNAs, with a mean fold down-regulation of 4.99 and 2.16 respectively at 48 and 30 days post-irradiation. These results were confirmed by qRT-PCR. Computa-
tional predictions identified collagen A1 as a potential target of miR-29b. Moreover, further evalu-
ation using tissue expression of miR-29b in different stages of wound healing revealed that miR-29b
expression in irradiated skin. These data suggest that miR29b plays a role in the regu-
lation of radiation-mediated skin fibrosis and might represent a potential therapeutic target for this
disabling condition.

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The presence of dermal papilla cells in bio-engineered skin substitutes improves the wound healing response in deep skin lesions in nude mice

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The bio-engineered composite skin could be a useful tool to treat deep and extensive skin injuries. We previously demonstrated the presence of human Dermal Papilla Cells (DPC) in a composite skin with Hair Follicle Stem Cells (HFSC), using acellular porcine dermis (APD) as scaffold, as a model system to elucidate the ionic and molecular mechanisms of the wound electric current. Using a systematic screen, electrophysiological techniques and molecular approaches, we identified an essential role for CIC in transport in the wound electric current and in healing. We first used broad-spectrum C1-channel blockers. Most of these blocked up to 50% of the cornea wound current as measured soon after wounding. When we did time-lapse experiments in the presence of C2a-activated C1-channel (C1C) blockers, measuring the wound current at regular time intervals after wounding, there was a drastic reduction of wound current (~80%). Whole-cell patch clamp recording showed that human corneal epithelial cells had a C2a-sensitive C1-current which was inhibited by ACA, a specific C2c blocker. ACA also blocked wound healing, but did not inhibit corneal epithelial cell (CEC) electrophysiology. ANOD1 (a C4c C1C) expression was upregulated after wounding, which indicated the presence of C1 channels in corneal epithelial cells, and ANOD1 knockout mice had significantly reduced wound currents. In slow healing wound, ANOD1 appeared to be downregulated in the cornea. Modulating CEC internal Ca2+ also altered wound currents. Those data also identify ANO1 as a novel role for C2a as in wound healing through wound electrical signal regulators. Key words: wound healing, electrical signal, ion channels. This work is supported by NIH 1R01EY019101.
Oligosaccharide modification by N-acetylglucosaminyltransferase-V promotes skin sclerosis by inducing macrophages to shift toward M2

M Terazawa,1,2,3 A Kato,1,2,3 H Muruta,1,2 E Miyoshi1,2 and I Katayama1 1 Dermatology, Osaka University Graduate School of Medicine, Suita, Japan and 2 Molecular Biology and Clinical Investigation, Okayama University Graduate School of Medicine, Okayama, Japan 1,2,3 Oligosaccharide modification by N-acetylglucosaminyltransferase-V (GnT-V) that catalyzes the formation of N-linked N-acetylglucosamine (GlcNAc) branches on N-glycans, is reported to be associated with various disease pathologies such as cancer metastasis, multiple sclerosis, and liver fibrosis. In this study, we describe the association of GnT-V with localized scleroderma. We found that GnT-V is highly expressed in fibroblasts and infiltrating cells in the skin sections from localized scleroderma patients. Most of the GnT-V-expressing cells were CD68+ and CD163+ positive macrophages. Macrophages play an important role in localized scleroderma. To know the role of GnT-V in scleroderma, we used murine model of scleroderma induced by bleomycin (BLM) injection. The expression of GnT-V was elevated in BLM-induced sclerotic skin. The most infiltrating skin sclerosis in GnT-V-deficient (GnT-V KO) mice. GnT-V KO mice were resistant to BLM-induced skin sclerosis with reduced collagen type I A1 content. Moreover, the number of macrophages in BLM-induced skin sclerosis was significantly fewer in GnT-V KO mice than in wild-type mice. Bone marrow-derived macrophages (BMDMs) from GnT-V KO mice were resistant to M2 shift induced by IL-4 demonstrated by significantly decreased arginase 1, Fizz1, and Ym1 expressions. Akt phosphorylation that is reported to play an important role in M2 shift was also decreased in the macrophages of the BLM-induced scleroderma model by BMDMs from GnT-V KO mice. Hence, GnT-V is an important molecule in both animal and human scleroderma. The inhibition of GnT-V expression may be a potential therapeutic strategy for scleroderma.

Superiority of hair follicle neogenesis in engineered human skin substitutes using early keratinocytes

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Hair follicle neogenesis (HF) has been a challenging goal for skin substitutes. The regeneration of HF has been limited, probably due to the use of late keratinocytes (KCs). We hypothesized that early KCs could enhance HF neogenesis. To test this hypothesis, we compared the effect of keratinocyte passage on HF neogenesis. Dermal equivalents were made with cultured dermal-epidermal composites; these were then grafted onto immunodeficient mice. Grafts using dermal equivalents expanded from 100,000 to 2,000,000 μm² and were resistant to M2 shift induced by IL-4 demonstrated by significantly decreased arginase 1, Fizz1, and Ym1 expressions. Akt phosphorylation that is reported to play an important role in M2 shift was also decreased in the macrophages of the BLM-induced scleroderma model by BMDMs from GnT-V KO mice. Hence, GnT-V is an important molecule in both animal and human scleroderma. The inhibition of GnT-V expression may be a potential therapeutic strategy for scleroderma.

Effect of human skin explants on the neurite growth and electrophysiological profile of the PC12 cell line

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The correlation between ultrasound findings and clinical assessment of pressure related ulcers: The Neilson and CRGP classification system. Patients who were classified as having a stage I or II pressure wounds based on clinical assessment and visual inspection. We postulate that patients presenting with clinically superficial stage I wounds will have a greater depth of injury than predicted. On admission, patients with sacral pressure ulcers were staged according to the NPUAP classification system. Patients who were classified as having a stage I or II pressure wounds or suspected deep tissue injury were assessed with high-frequency (12-MHz) ultrasound (US) to identify any evidence of injury to the deep tissue. Those patients classified as having stage III or IV were excluded from the study. The study included 17 patients undergoing US for pressure related ulcers of the sacrum; 9 patients with Stage I pressure ulcers and 8 patients classified as having suspected deep tissue injury. In all 9 patients with clinically superficial stage I wounds, the US demonstrated evidence of injury to the deeper tissue layers. For the 8 patients classified as suspected deep tissue injury, the US also revealed abnormal findings representing deep tissue injury. The abnormal sonographic signs indicating deep tissue injury included loss of epidural dermal interface, disrupted dermis, and hypochoic hyperechoic layered structures. The current staging system used by the NPUAP has expanded to include suspected deep tissue injury as an additional stage. In patients with suspected deep tissue injury, we have found US to be a reliable diagnostic tool that could be used for clinical assessment of deep tissue injury. Interestingly, for the stage I patients who appeared clinically superficial, the US revealed evidence of associated deep tissue injury. This suggests that pressure wounds classified as superficial may have a deeper tissue damage component.
The microbially colonizing diabetic foot ulcers is associated with glyceric control, ulcer depth, and duration. SE Gardner, SL Hills, K Heilmann and IA Green. 1 College of Nursing, University of Iowa, Iowa City, IA; 2 Carver College of Medicine, University of Iowa, Iowa City, IA; 3 Department of Dermatology, University of Pennsylvania, Philadelphia, PA. Microbial colonization and/or infection are believed to underlie delayed healing in recalcitrant wounds, including diabetic foot ulcers (DFU). However, little is known of those clinical factors that may influence the wound environment and thus various dimensions of the colonizing microbiota. We isolated the microbially colonizing neuropathic, non- ischemic DFUs in 52 individuals by deep sequencing of the bacterial-specific 16S ribosomal RNA gene. Quantitative cultures, the standard of care in the clinic, was underrepresented and microbial diversity and distribution. DFUs partitioned into three clusters differentiated by microbial diversity and composition. We identified several clinical features associated with features of the DFU microbiome including glyceric control, ulcer depth, and duration. Hemoglobin AIc (HgA1c) value, a measure of glyceric control, significantly associated with DFU cluster (P<0.01), with highest HgA1c levels partitioning to the Streptococcus and Staphylococcus group, whereas positively correlated with relative abundance of anaerobic bacteria (P=0.03, P=0.018), but negatively correlated with relative abundance of Staphylococcus (P=0.47, P=0.0005). Greater number of bacterial species and high relative abundance of Gram-negative bacteria were seen to be associated with DFUs. To develop a better understanding of the relationship between the colonizing microbiota and wound healing, we investigated the temporal and spatial distribution of microbial communities in DFUs with different clinical characteristics. In conclusion, our data suggest that DFUs with different clinical characteristics have distinct microbial communities, which may influence wound healing. Further studies are needed to determine the role of the colonizing microbiota in the delayed healing of DFUs and to develop strategies to improve wound healing in these patients.
C7 plays a dual role in skin wound healing

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Recessive dystrophic epidermolysis bullosa (RDEB) is a heterogenous skin fragility disorder caused by mutations in the COL7A1 gene encoding collagen VII (C7), a protein required for secure attachment of epithelial cells to the dermis. Patients suffer from a constant wound burden with repetitive ulcerations. The pathogenesis of skin wounds involves inflammation, and it is known that epithelial keratinocytes are key players in the process of wound healing. However, their role in this process is not yet completely understood. We recently reported that C7 is involved in keratinocyte migration. Here, we investigated whether C7 is also involved in epithelial keratinocyte migration, and we also examined its role in the regulation of keratinocyte migration in RDEB.

We observed that C7 is required for keratinocyte migration, and that a deficiency in C7 leads to a reduced ability of keratinocytes to migrate. We also showed that a deficiency in C7 leads to an increased expression of the pro-inflammatory cytokine IL-1β, which is known to play a role in the regulation of keratinocyte migration. These findings suggest that C7 is involved in the regulation of keratinocyte migration and that a deficiency in C7 leads to an impaired ability of keratinocytes to migrate. This study provides new insights into the role of C7 in the regulation of keratinocyte migration and may contribute to the understanding of the pathogenesis of skin wounds in RDEB.
VEGF promotes cutaneous wound healing via VEGFR-1 signaling in keratinocytes and macrophages
K Johnson, M Lachev and T Wilges Pathology, The Ohio State University, Columbus, OH

Vascular endothelial growth factor (VEGF) plays an important role during wound healing by activating VEGF receptors (VEGFR) on endothelial cells, which stimulates angiogenesis. Interestingly, VEGFR-1 has now been described on other cell types critical for wound healing, including keratinocytes and macrophages. This suggests that VEGF may stimulate repair through angiogenic and non-angiogenic mechanisms. The purpose of this study was to determine whether VEGF directly affects keratinocytes and macrophages during wound repair. Healing was assessed in full-thickness excisional wounds from two unique conditional knockout (KO) mouse strains in which VEGFR-1 was ablated in either keratinocytes or macrophages. A significant delay in wound closure was observed in mice lacking VEGFR-1 in epidermal keratinocytes (epiKO). Wounds from epoKO mice contained fewer macrophages and healed more slowly than control wounds. Strikingly, only 20% of epoKO wounds closed by Day 14 compared to 80% of control wounds, suggesting that VEGF may mediate pro-inflammatory mediator production by keratinocytes and directly promote reepithelialization by keratinocytes. Healing was also examined in mice lacking VEGFR-1 in macrophages (macKO). Significantly fewer macrophages were obtained in wounds from macKO mice compared to controls, indicating that VEGF acts as a macrophage chemotaxant during repair. Only 15% of the macKO wounds showed complete closure at 5 days compared to 42% of control wounds. Reduced macrophage recruitment in macKO mice contributed to the delay in wound closure, as macrophages produce a variety of factors that affect reepithelialization by keratinocytes. Overall, these results suggest that in addition to stimulating angiogenesis, VEGF affects wound healing by acting through VEGFR-1 on keratinocytes and macrophages. These studies provide new information about how VEGF contributes to dermal repair, which could be useful for the development of new strategies to prevent chronic wounds and improve healing.

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1515 Role of kallikrein 6 during epidermal regeneration after glucocorticoid-induced cutaneous atrophy
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One of the major adverse effects of glucocorticoid therapy is cutaneous atrophy often followed by the development of resistance to steroids (tachyphylaxis). Previously we showed that within two weeks of topical fluocinolone acetonide (FA) treatment, interfollicular mouse keratinocytes acquired resistance to the anti-proliferative effects of this glucocorticoid. To search for potential mechanisms by which keratinocytes regain their ability to proliferate, we performed extensive DNA array analyses focusing on genes that were activated after a 2 week treatment with steroids. Kallikrein 6 (Klk6), a serine protease known to enhance keratocyte proliferation and migration both in vitro and in vivo was the most up-regulated (18-fold) gene. We confirmed the increased expression of Klk6 by RT-PCR and immunostaining. Interestingly, Klk6 was expressed in single keratinocytes in the suprabasal layer of the atrophic mouse epidermis, and these Klk6+ keratinocytes were adjacent to BrdU-labeled keratinocytes. Epidermal induction of Klk6 was also noted after application of glucocorticoid clobetasol propionate to human skin. We identified multiple putative glucocorticoid response elements in the Klk6 promoter. The expression of Klk6 was also examined in fetal wounds. In this model, wounds created at embryonic day 15 (E15) resulted in accelerated epithelial closure. Thus, this study demonstrates that BMPs negatively regulate the length and area of hyperproliferative epithelium compared to controls. However, cell proliferation in regenerating epidermis was not correlated with TG mice versus the controls. In contrast, TG mice showed significant increase in the number of TUNEL+ apoptotic cells in the hyperproliferative epithelium compared to controls. Also, primary keratinocytes isolated from TG mice showed significant increases in cell migration compared to TG MCSAs as measured by in vitro wound healing and microray data revealed that a large number of the motility-associated genes encoding the essential components of the cell migration machinery (mosmos, tropomin, etc.) serve as direct Satb1 targets in these cells. Thus, Satb1 downregulation likely serves as a part of the protective response mechanism controlling epidermal regeneration during wound healing, while Satb1 targeting might be used as a novel approach to improve skin regeneration in chronic wound conditions.

1517 Light emitting diode generated red light modulates kldeloid-derived fibroblast proliferation and migration speed
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Keloids are a disorder characterized by increased fibroblast proliferation and extracellular matrix deposition. Cultured keloid-derived fibroblasts also demonstrate increased migration speed compared to fibroblasts derived from normal human skin. We previously found that light emitting diode-generated red light (LED-RL) can modulate normal human skin fibroblast proliferation. Here we hypothesized that LED-RL can modulate keloid-derived fibroblast proliferation and migration speed. To test this hypothesis, two different keloid-derived fibroblast lines were irradiated with LED-RL, each matched with a temperature regulated “bench control plate” (BCP), to ensure that the measured effect was a result of LED-RL treatment and not due to other environmental factors. LED-RL at fluences of 320 J/cm2 and 480 J/cm2 significantly decreased cell proliferation at 48hrs post irradiation (14 – 29% decrease from control, temperature-matched BCP cells, p<0.05), with no significant difference in cell viability, as measured by trypan blue exclusion. Keloid-derived fibroblasts also demonstrated increased migration speed compared to normal fibroblasts as measured by wound healing in vitro.

1518 Genomic organizer and At-rich binding protein Satb1 inhibits epidermal regeneration during wound healing via modulation of cell migration and apoptosis
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1519 IL-33 is upregulated after injury and stimulates inflammation during repair
B Woll and T Wilgus Pathology, The Ohio State University, Columbus, OH

Inflammation plays an important role in repair by eliminating potential pathogens from the wound. However, inflammation can also delay healing and stimulate scar formation in some cases. Despite the importance of inflammation in the healing process, the exact mechanisms by which inflammation is initiated after injury are not fully understood. Recently, a role for interleukin-33 (IL-33) has emerged in the skin. IL-33 is a member of the IL-1 cytokine family and has been described as a cytokine that mediates inflammation. There are few effective treatment options for keloids and other cutaneous fibrotic diseases. To test these hypotheses, two different keloid-derived fibroblast lines were irradiated with LED-RL, hypothesized that LED–RL can modulate keloid-derived fibroblast proliferation and migration speed.

1520 Bone morphogenetic protein signalling regulates keratinocyte proliferation and migration during wound healing in murine and human skin
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Bone morphogenetic proteins (BMPs) and their receptors (BMPRs) regulate tissue development and remodelling; however, their role in wound healing is unclear. To study the role of BMPs in skin repair, we used transgenic mice overexpressing the BMP antagonist noggin or BMP downstream component BMP4, as well as a human ex vivo wound healing model. K14 promoter as in vivo models, as well as a human ex vivo wound healing model. K14-Noggin mice exhibited accelerated wound healing, associated with increased keratinocyte proliferation at the wound margin and a hyper-proliferative wound epithelium, as well as increased wound capillary density versus the corresponding controls. In contrast, K14-Sma1 mice exhibited retarded wound healing and significantly reduced wound epithelial surface area compared to controls. qRT-PCR showed down-regulation of BMP4 and 6 up-regulation in murine wounded skin as well as down-regulation of the BMP receptor ligands (BMP2/4/7). Human and mouse keratinocyte proliferation was impaired after treatment with BMP4/7 as assessed using flow cytometry, whereas treatment with BMP antagonist noggin increased cell proliferation. At 48hrs, the mRNA levels of IL-33 were not significantly increased in K14-Noggin mice. We demonstrate that BMP4/7 as assessed using flow cytometry, whereas treatment with BMP antagonist noggin increased cell proliferation. Satb1 downregulation likely serves a part of the protective response mechanism controlling epidermal regeneration during wound healing, while Satb1 targeting might be used as a novel approach to improve skin regeneration in chronic wound conditions.

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1516 Generation of 3D full-thickness skin equivalents exclusively from human induced pluripotent stem cell (iPSC)-derived keratinocytes and fibroblasts
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Cell-based therapies are limited by the number of undeferentiated cells available and/or by immune rejection. Generation of iPSCs can provide unlimited source of immunocompatible, patient-specific cells for medical applications, thus circumventing the obstacles encountered by adult and embryonic stem cells. However, the potential for iPSCs in regenerative medicine is still being realized. Here, we have generated full-thickness 3D skin equivalents using exclusively iPSC-derived cells. iPSCs were generated from human dermal fibroblasts isolated from foreskin, by exoge- nous expression of 4 transcription factors, Oct4, SOX2, MYC, and KLF4. Keratinocytes were dif- ferentiated from iPSCs using the method developed in our lab, by treatment with RA and BMP4. Fibroblasts were derived from iPSCs using ascorbic acid and TGFβ2 before culturing in DMEM supplemented with ascorbic acid and 20% horse serum. These cells assumed fibroblast-like morph- ology and expressed typical CD surface markers such as CD10, CD44, CD71 and CD90. To generate 3D skin equivalents, iPSC-derived fibroblasts were embedded in 3 mg/ml of rat tail type 1 collagen and cultured for 7 days before applying iPSC-derived keratinocytes on top of fibroblast- containing collagen gels. Epidermal differentiation was facilitated by lifting gels to the air interface. Matrigel and oin staining revealed a well-differentiated epidermis with distinct basal, spin- dal and granular layers and a stratum corneum, anucleate. Furthermore, SEM and confocal micro- imaging revealed that a large number of the motility-associated genes encoding the essential components of the cell migration machinery (mosmos, tropomin, etc.) serve as direct Satb1 targets in these cells. Thus, Satb1 downregulation likely serves a part of the protective response mechanism controlling epidermal regeneration during wound healing, while Satb1 targeting might be used as a novel approach to improve skin regeneration in chronic wound conditions.
1521 TNS4 delayed wound healing through actin cytoskeletal regulation
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Tensin proteins are known to play a pivotal role in cell migration, wound healing and tumorigenesis. TNS4 is one of the four tensin family members, but little is known about its role in skin epidermal migration and wound healing. In this study, TNS4 was over-expressed in primary cultured normal human epidermal keratinocytes (NHEK) in vitro or on epidermis in vivo using a GFP tagged adenoviral over-expression system. We examined the role of TNS4 in the skin through a wound healing assay. In the TNS4 over-expressed NHEK, TNS4 was observed in the peripheral cytoplasm, and the morphology and the size of the keratinocytes differed from that of the GFP controls. Consistent with the morphological change, F-actin polymerization was decreased where TNS4 was over-expressed on immunocytofluorescent staining. When in vitro scratch assay was performed, scratched area was remained significantly larger in the TNS4 over-expressed cell layer after 48 hours compared to the GFP control group. (59% VS. 26%, respectively, n=3, p<0.05) indicating that cell migration was diminished by TNS4 over-expression. Cdc42 have well known associations with F-actin polymerization and cell migration. We found that Cdc42 protein level was reduced by 50% when TNS4 was over-expressed, in addition to the downstream signals of Cdc42 being reduced. Cdc42 level was restored when treated with a proteasome inhibitor was treated. When TNS4 was over-expressed, the proportion of S phase was reduced in TNS4 over-expressed group. Finally, we confirmed a delay in wound healing in vivo using mice back skin with punch injury. Four days after wounding, the injured area was 1.7-fold (p<0.05) wider in the TNS4 over-expressed group compared to the GFP control group. In conclusion, our study suggested that TNS4 over-expression triggers Cdc42 degradation through a proteasome pathway and eventually affects the cytoskeletal system, which leads to aberrant cell migration, proliferation, and delayed wound healing.

1523 Expression of either microRNA-198 or FSTL1 from a primary transcript is controlled by TGF-β and KSRP for efficient wound healing
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Wound healing requires the integrated regulation of complex biological events including cell migration, proliferation and extracellular matrix remodeling, globally stimulated by TGF-β and other growth factors. We report a novel post-transcriptional switch that dictates the spatio-temporal and mutually exclusive expression of two alternative gene products from a single transcript. In un wounded normal skin expression of exonic microRNA-198 (miR-198), located in the 3′-untranslated region of follistatin-like-1 (FSTL1) mRNA is observed. Upon wounding miR-198 expression is shut down and expression switches to the linked open-reading-frame (ORF) of FSTL1. We demonstrate that binding of a KH-type splicing regulatory protein (KSRP) to the primary transcript determines the fate of the transcript and is essential for the processing of miR-198. TGF-β-signaling switches off miR-198 expression by down-regulating KSRP, which promotes FSTL1 protein expression. We also observed that FSTL1 expression promotes keratinocyte migration, whereas miR-198 expression has the opposite effect by targeting and inhibiting genes important for migration. A clear inverse correlation between the expression pattern of FSTL1 (pro-migratory) and miR-198 (anti-migratory) highlights the importance of this regulatory switch in controlling context-specific gene expression to orchestrate wound re-epithelialization. The deleterious effect of failure of this switch is apparent in non-healing chronic diabetic ulcers, where expression of miR-198 persists, FSTL1 is absent and keratinocyte migration, re-epithelialization and wound healing all fail to occur.

1522 Development of ECM-based biohybrid skin substitutes
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Objective: To utilize discarded adipose tissues as waste-to-resource materials for the development of in vitro biohybrid skin substitutes containing natural biological cues. Currently, adipose tissue is often discarded after plastic and reconstructive surgery in the form of lipoaspirates and grafts. A chemical-free decellularization method was used to obtain ECM from the human adipose tissues. The whole decellularization process used to treat the liposprirate material takes place in over an hour as compared to days taken by the commonly-used chemical and hybrid methods. H&E staining results showed no evidence of viable nuclear material, whilst the amount of dsDNA left in the dry ECM was 47.1 ng/ml. SEM analysis further confirmed the removal of both cells and lipids from the remaining fibrous ECM material. Besides, immunostaining results showed that key structural proteins such as collagen type I (Col1), collagen type IV (ColIV) and laminin remained intact in the ECM. Angiopoietin-like-4 (ANGPTL4), a matricellular protein that is implicated in wound healing and angiogenesis, is also bound intact in the ECM obtained using our chemical-free decellularization method. The successful coupling of ECM with alginate was confirmed by the presence of amide I band (at 1642 cm-1) and amide II band (at 1523 cm-1) in FTIR spectrum. Alginate scaffolds after 60 min of second cross-linker treatment had relatively larger pores and thicker inter-pore connectivity compared to those without second cross-linker treatment. It was observed that the size of the scaffolds decreased with increasing duration of second cross-linker treatment. Overall, this biohybrid scaffold is a stepping-stone towards the development of tunable acellular scaffolds with cellular benefits without the need for additional biologics.