26.01 - ASC20151280

Dipeptidyl peptidase-4 Inhibition Promotes Wound Healing in Murine Models of Type 1 and 2 Diabetes

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Scientific Area: Wound Healing/Fibrosis
Clinical Area: Cross-cutting or N/A

Introduction: Chronic diabetic wounds are thought to result from impairments in the cellular and molecular mechanisms of wound repair in diabetic patients. Clinically, these wounds can result in significant disability, amputation, and increased mortality. Poor neovascularization in response to ischemia has been discovered to be fundamental to this problem, resulting from a high glucose induced defect in the transactivation of hypoxia-inducible factor-1α (HIF-1α). This leads to impairment of HIF-1α mediated expression of vascular endothelial growth factor (VEGF) and stromal-derived factor-1 (SDF-1). In particular, diabetic patients are known to be deficient in SDF-1, and it has been shown that in non-diabetic cells inhibiting DPP-4, which cleaves SDF-1, enhances the chemotaxis of murine and human HSCs and hematopoietic progenitor cells in vitro via SDF-1 induction. To explore the potential therapeutic benefits of DPP-4 inhibition for diabetic wounds, we utilized the DPP-4 inhibitor MK0626 and tested its effect on wound healing and cell behavior.

Methods: Wild-type mice were either treated with streptozocin or fed a high fat diet to induce type 1 and 2 diabetes, respectively, which was confirmed via glucose tolerance test. Type 1 and type 2 diabetic mice were treated for 6 weeks with either: vehicle (high fat chow), glipizide (hypoglycemic agent), or MK0626. Humanized excisional wounds were subsequently created on the dorsum of treated type 1 and type 2 diabetic mice, with wounds being photographed and assessed at two-day intervals. Tissue was harvested for histology and qRT-PCR.

Results: MK0626 significantly accelerated wound healing compared to both control and glipizide groups (*p= <0.05). This effect became evident by day 4, with significantly reduced mean wound area relative to original size (MK0626 = 79%, Control = 90%, Glipizide = 87%, *p= <0.05). qRT-PCR demonstrated increased transcription of SDF-1 (*p= <0.05), and vascular endothelial growth factor (VEGF) (*p= <0.05) in MK0626 treated v control mice. Immunohistochemistry studies demonstrated increased expression of SDF-1 (*p=0.008) and CD31 (*p=0.02) in MK0626 v control groups.

Conclusion: DPP-4 inhibition has the potential to play a pivotal role in diabetic wound healing. The administration of MK0626 enhances wound healing more effectively than reducing hyperglycemia alone. Furthermore, a low concentration of MK0626 (1mg/kg/BW in chow) seems to be the optimal dosage for oral administration.
MK0626 Improves Diabetic Wound Healing in Type II Diabetic Mice

A

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<th>Day 0</th>
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<th>Day 12</th>
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Healed Day 20
Healed Day 18
Healed Day 18
Healed Day 16
Healed Day 16
Healed Day 16

B

Wound Area (%) vs. Time (Days)

- Control
- Glipizide
- MK0626

C

Days vs. Control, Glipizide, MK0626

* Indicates significant difference
**26.02 - ASC20150133**

**SDF-1α attenuates diabetic wound inflammation through modulation of miR-146α expression**

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**Scientific Area:** Wound Healing/Fibrosis  
**Clinical Area:** General Surgery

**Introduction:** Impaired wound healing represents a significant complication of diabetes. The etiology of this wound healing impairment is multifactorial and includes an increased and chronic inflammatory response. We have previously shown that murine diabetic wounds are deficient in SDF-1α and that treatment of diabetic wounds with SDF-1α can improve healing, however, the mechanisms of this correction are not well characterized. MicroRNAs (miRNAs) are novel RNA molecules that regulate the translation of mRNAs at the post-transcriptional level. In particular, microRNA-146a (miR-146a) has been shown to be a key repressor of the inflammatory response by targeting interleukin-1 receptor associated kinase (IRAK1) and tumor necrosis factor receptor associated factor 6 (TRAF6) and repressing NFκB. We have recently shown that miR-146a expression is decreased in murine diabetic wounds. We hypothesized that the improved diabetic wound healing following treatment with SDF-1α is due, in part, to correction of the dysregulated miR-146a gene expression resulting in decreased inflammatory response.

**Methods:** To test this hypothesis, 8mm full-thickness wounds were created on the flank of diabetic (Db/Db) and non-diabetic (Db/+) mice with a dermal punch instrument. At the time of wounding, the wounds were treated with an intradermal injection with either 108 plaque-forming units (PFU) of a lentivirus containing the SDF-1α or GFP transgene. The wounds were harvested 7 days after injury and processed for histology and isolation of total cellular RNA. In order to examine the role of the fibroblast in the dysregulation of the inflammatory response, we isolated diabetic and non-diabetic dermal fibroblasts and treated them with either 106 PFU of a lentivirus containing the SDF-1α or GFP transgene. Gene expression was analyzed using Real-time PCR analysis.

**Results:** At 7 days, diabetic wounds treated with lenti-SDF-1α exhibited a significant decrease in wound surface area compared to lenti-GFP treated wounds. Diabetic wounds treated with SDF-1α also demonstrated a significant increase in miR-146a expression, and a significant decrease in the miR-146a targets IRAK-1 and TRAF-6, compared to GFP treated diabetic wounds. At the cellular level, MiR-146a was significantly down-regulated in diabetic fibroblasts at baseline, compared to non-diabetic fibroblasts. In addition, SDF-1α treatment corrected the miR-146a gene expression to levels similar to the non-diabetic fibroblast.

**Conclusion:** SDF-1α mediated correction of the diabetic wound healing impairment is due, in part, to correction of dysregulated microRNA-146a expression. Increased expression of miR-146a may result in decreased inflammatory response and less oxidative stress resulting in improved healing. Further studies are needed to define the role of abnormal miRNA regulation in the pathogenesis of the diabetic wound healing impairment.
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Accelerated Myocutaneous Revascularization Following Graded-Isschemia in db/db Mice
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Scientific Area: Wound Healing/Fibrosis
Clinical Area: Trauma/Critical Care

Introduction: Murine models have provided valuable insight into the pathogenesis of both diabetes and chronic wounds. The db/db mouse possesses a spontaneous mutation in the leptin receptor gene resulting in obesity, hyperglycemia, hyperinsulinemia, hypercholesterolemia, and insulin resistance. This strain is characterized by impaired epithelialization of excisional wounds, with little known about wound neovascularization.

Methods: A cranial-based, peninsular-shaped myocutaneous flap was surgically created on the dorsum of C57BL6 (wild-type) and db/db mice (n=16 total; 5 mice per operative group and 3 unoperated mice per group as controls). Planimetric analysis of serial digital photographic images was utilized to determine flap viability in wild-type and db/db mice. Real-time myocutaneous flap perfusion and surface temperature were determined by laser speckle contrast and thermal infrared imaging respectively. Mice with surgical flaps were sacrificed on postoperative day 10. Image analysis of CD-31 immunostained sections confirmed flap microvascular density and anatomy. Quantitative RT-PCR was performed on nonoperative back skin and postoperative flap tissue specimens to determine local gene expression.

Results: Day 10 planimetric analysis revealed a mean flap viability of 95% in db/db mice compared to 79% in wild-type mice, and 60% of wild-type mice developed distal flap dehiscence not evident in db/db mice. Over 10 days, laser speckle contrast imaging documented markedly increased perfusion at all times points (p<0.001) with functional revascularization to supranormal perfusion in db/db flaps. In contrast, wild-type flaps displayed expected graded flap ischemia with failure to return to baseline perfusion during the postoperative period. Thermal infrared imaging documented complementary spatiotemporal assessment of thermal-metabolic tissue state. Immunostaining confirmed significant differences in preoperative and postoperative microvascular density (mean post-op distal vessel count 60 ± 8 versus 80 ± 15 vessels/mm² for wild-type and db/db mice respectively, p=0.018; mean post-op distal vascular surface area 4108 ± 95 versus 8250 ± 795 µm²/mm² for wild-type and db/db mice respectively, p<0.001). Finally, quantitative RT-PCR demonstrated statistically significant differences in angiogenic gene expression between wild-type and db/db mice at baseline (unoperated) and at day 10.

Conclusion: In a graded-ischemia wound healing model, accelerated myocutaneous revascularization and improved wound healing were evident in db/db mice compared to wild-type controls. Gene expression analysis reveals the db/db mouse may be “primed” for wound neovascularization and warrants further investigation into the role of the leptin-leptin receptor axis in wound angiogenesis.
Effect of Stretch On Extracellular Matrix And Morphology Of Fibroblasts in Regenerative Wound Healing

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Scientific Area: Wound Healing/Fibrosis
Clinical Area: General Surgery

Introduction: Mid gestation fetal skin heals without scar and is characterized by negligible resting tension and distinct extracellular matrix (ECM) with elevated levels of hyaluronan (HA) produced by fetal fibroblasts. In contrast, adult skin is characterized by relatively low levels of HA, and much higher resting tension and scar formation. Of note, wounds of a critical size even in the fetus heal with a scar suggesting a role for biomechanical forces in the regulation of the fetal regenerative phenotype. There is little data that examined the interaction of mechanical stress and regulation of the ECM, specifically HA synthesis. Taken together, we hypothesize that higher mechanical tension may alter the fibroblast regulation of HA synthases (HAS1-3).

Methods: Primary murine adult fibroblasts (AFb) and fetal fibroblasts (FFb) were cultured with +/- mechanical stretch for 24 hours. Static tension at 58 KPa (resting tension of adult mouse skin) was applied using flexcell apparatus to induce tonic stretch. Stretch induced phenotypic changes in FFb and AFb were assessed by changes in PCM (particle exclusion assay), HAS1-3 synthases (qPCR) and cell morphology and cytoskeleton changes (a-SMA Immunohistochemistry). Data presented as average+/-SD, n=4/group, p values by t-Test or ANOVA.

Results: AFb and FFb demonstrate differential cell responses to biomechanical stretch. Under static condition, AFb had significantly lower HAS1 (AFb 1.4+/-.4 vs FFb 4.6+/-.1, p<0.01) and HAS2 (AFb 1.2+/-.25 vs FFb 2.7+/-.8, p<0.01) gene expression that encodes for fetal-like high molecular weight hyaluronan, and smaller PCM (AFb 1.84+/-.08 vs FFb 2.78+/-.14; p<0.001). AFb also demonstrated thicker a-SMA fibers compared to FFb, but the fiber orientation appeared random in both cell types. 24 hours of mechanical stretching resulted in the loss of FFb phenotype with a significant decrease in HAS1 (FFb+stretch 1.0+/-.35 vs FFb 4.6+/-.1, p<0.01) and HAS2 (FFb+stretch 1.0+/-.2 vs FFb 2.7+/-.8, p<0.01) gene expression and a significant reduction in PCM formation (FFb+stretch 1.98+/-.15 vs FFb 2.78+/-.14, p<0.01) in FFb, to levels similar to the AFb under static condition. Stretching increased HAS1 expression in AFb (AFb+stretch 3.0+/-.6 vs AFb 1.4+/-.4, p<.05), but had no effect on HAS2. Interestingly a-SMA staining demonstrated that stretching resulted in thickening of the stress fibers in FFb, but not AFb, and a trend toward reorientation of actin fibers in the strained cells perpendicular to the direction of stretch in both AFb and FFb.

Conclusion: Our data suggest that biomechanical forces may have a significant role in influencing the dermal fibroblasts' morphology and their regulation of the ECM in the fetal and adult wound healing phenotype. Understanding the contribution of mechanical environment via morphological and phenotypic alterations may yield novel therapeutic targets in recapitulating fetal regenerative healing in postnatal tissues.
GelE/sprE are critical for Enterococcus faecalis-induced anastomotic leak in a rat model

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Scientific Area: Wound Healing/Fibrosis
Clinical Area: General Surgery

Introduction: Anastomotic leak following colorectal surgery is a dreaded complication of which the cause remains unknown. Our laboratory has recently established that high collagenase producing strains of Enterococcus faecalis (E. faecalis) “bloom” in the gut following anastomotic injury and are both necessary and sufficient to cause anastomotic leak in rats. Here we performed a mutational analysis to define the roles of gelE and sprE – co-regulated genes that control: adherence, penetration and collagenase production – on anastomotic leak. We used a double knockout mutant lacking gelE and sprE derived from E. faecalis V583 (a vancomycin-resistant clinical isolate). We hypothesize that mutants deficient in both genes (ΔgelEΔsprE) would be attenuated in their capacity to cause leak in rats.

Methods: Adult rats (n=24) underwent colon resection followed by recto-sigmoid anastomosis. Following anastomotic construction, 5cc of 10⁷ CFU/ml of either the double mutant deficient in both gelE and sprE (ΔgelEΔsprE), or the mutant complemented with gelE and sprE (ΔgelEΔsprE/gelE+sprE- termed VT07) were administered via rectal enema to inoculate the anastomosis. On postoperative day six, all rats were sacrificed and evaluated for evidence of anastomotic leak via previously established criteria. Anastomotic tissues were harvested and separated into mucosal, serosal, and perianastomotic samples for bacterial species identification via culture and phenotype analysis (collagenase activity).

Results: Anastomotic leakage was observed to be significantly greater in rats rectally inoculated with the complemented strain VT07 that produces high collagenase compared to its matched double mutant ΔgelEΔsprE that produces no collagenase (p<0.01). Leaks were characterized by dense perianastomotic adhesions, perianastomotic abscesses, and occasional gross anastomotic dehiscence. The most severe leaks were identified to be associated with high adherence of E. faecalis to the mucosa, penetration into the serosa, high collagenase activity, and culture positivity of perianastomotic tissues. Dense mucosal colonization alone was not associated with leak.

Conclusion: High collagenase producing E. faecalis present at and within anastomotic tissues appears to play a critical role in the pathogenesis of anastomotic leak. Although various genes are likely to confer its pathogenic potential at healing anastomotic tissues, we show in this study that gene products and pathways regulated by the gelE/sprE system that control adherence, penetration, and collagenase/protease production are likely to play a key role. Because E. faecalis is a ubiquitous commensal organism in humans, and only certain strains of it are associated with anastomotic leak, a more complete understanding of the microbial pathogenesis and phenotypic expression could enable us to better predict leak and thus improve patient management.
Adipose-Derived Stem Cell-Seeded Hydrogels Increase Progenitor Cell Recruitment and Functionality

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Scientific Area: Wound Healing/Fibrosis
Clinical Area: Other

Introduction: Biomaterials that promote neovascularization represent a promising treatment of chronic wounds, particularly in combination with stem cells. Adipose-derived mesenchymal stem cells (ASCs) are of particular interest, due to their pro-regenerative function and ease of harvest. Our laboratory has demonstrated the benefits of delivering ASCs seeded on a pullulan-collagen hydrogel bioscaffold, resulting in upregulation of genes related to stemness and neovascularization and accelerated wound closure in vivo. More recently, it has been shown that mesenchymal stem cells (MSCs) enhance the recruitment of endogenous progenitor cells, likely through cytokine secretion. Therefore, we investigated the recruitment of endogenous progenitor cells by ASCs seeded on our hydrogel bioscaffold.

Methods: ASCs were either seeded into hydrogels or plated in standard growth medium. After 48 hours of incubation in hypoxia, conditioned medium (CM) from the ASC-seeded hydrogels and from the plated ASCs was harvested. The effects of CM from ASC-seeded hydrogels on the functionality of bone-marrow mesenchymal progenitor cells (BM-MPCs), a cell population defined by our laboratory to be crucial to neovascularization, were assessed using qPCR, ELISAs, immunocytochemistry to measure cell proliferation, a migration assay, and a tubulization assay. In vivo, we parabiosed green-fluorescent protein positive (GFP+) donor mice and wild-type recipient mice. An excisional wound model on the recipient mouse was treated with a control saline injection, injection of ASCs, or ASC-seeded hydrogels, and wounds were harvested four days post-wounding for fluorescence-activated cell sorting (FACS) and microfluidic single-cell analysis.

Results: In vitro, we found that ASC-seeded hydrogel CM significantly upregulated the functionality of BM-MPCs. In particular, BM-MPCs exposed to ASC-seeded hydrogel CM displayed a significant increase of 44% in cell migration (p=0.019) and a fourfold increase in cell proliferation (p=0.0027). Furthermore, qPCR and ELISAs revealed the upregulation of several angiogenic genes, including Hgf and Mmp3, and a significant increase in tubulization of BM-MPCs exposed to ASC-seeded hydrogel CM (p=0.045). In vivo, microfluidic single-cell analysis identified a subpopulation of GFP+/Lineage-/CD45- cells, putative BM-MPCs, defined by the expression of pro-vasculogenic genes, including Ang, Cd248, and Sca1, that was increased in wounds treated with ASC-seeded hydrogels (45.8% of GFP+/Lin- cells) compared to injected ASCs and PBS controls (32.7% and 22.8% of GFP+/Lin- cells), respectively. FACS quantification of GFP+/Lin-/CD45-/Sca1+ cells within the wounds demonstrated an increase of 38% in recruited BM-MPCs when wounds were treated with ASC-seeded hydrogels compared to ASC injection.

Conclusion: ASC-seeded hydrogels upregulate BM-MPC functionality, and enhance BM-MPC recruitment, to effect greater neovascularization and accelerate wound healing.
26.07 - ASC20150428

Nanocarrier-decorated Mesenchymal Stem Cells for Therapeutic Wound Healing and Angiogenesis
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Scientific Area: Wound Healing/Fibrosis
Clinical Area: Other

Introduction: Mesenchymal stem cells (MSC) carry high therapeutic potential for tissue regeneration. However, targeted delivery of therapeutic MSC to injured tissue remains a challenge. We are aimed to develop a safe and targeted cell delivery method for therapeutic administration of MSC to injured tissues to achieve enhanced wound healing and neovascularization. Herein, we present a novel cell delivery platform by coating the surface of MSC with nanocarriers composed of nanoparticle–adhesion molecule complex (NAMC). These nanocarriers guide the coated MSC to their destination via molecular recognition and association with their counterpart adhesion molecules, which are highly or selectively expressed on the injured tissue, and execute their therapeutic roles.

Methods: Murine bone marrow-derived MSC, which were obtained from ROSA 26-LacZ⁺ mice and pre-labeled with Luciferase 2 (Luc2⁺) gene, were coated with nanocarriers (NAMC) or control nanoparticle-albumin complex (NAC). 1x 10⁶ NAMC- versus NAC-decorated MSC were administrated through either systemic (i.v.) or local (wound tissue injection) approach to recipient mice on which 6-mm full thickness dorsal skin wounds were created (n=6/group). An extra group of wounded mice were treated with saline as baseline control. Bio-distribution and selective wound tissue homing of administrated Luc2⁺ MSC were detected by IVIS (In Vitro Image System) at various time points. Increased homing of infused circulating NAMC-decorated MSC to wound tissues was validated by X-gal staining of LacZ⁺ MSC in wound tissues. Wound healing rate was calculated based upon daily digital photograph of wounds. Neovascularization was assessed by either Dil-perfusion and wound tissue confocal microscopy (for local administration) or immunohistochemistry analysis of wound tissue sections (for systemic administration).

Results: Whole-body IVIS scan showed significantly increased Luc2⁺ NAMC-decorated MSC within wound tissues, particularly on day 8 (~10-fold increased bioluminescence signal, p=0.029). Intravenously infused NAMC-decorated MSC selectively homed to wound tissues, but not other organs. Increased homing of intravenously infused NAMC-decorated MSC to wound tissues was demonstrated by X-gal staining of wound tissues. Wound healing rate and neovascularization were significantly increased by both systemic (p<0.01) and local (p<0.01) administration of NAMC-decorated MSC in comparison with NAC-decorated control MSC.

Conclusion: We demonstrated the feasibility and efficacy of a novel form of nanocarrier for targeted delivery of therapeutic stem cells to wound tissues in mouse models for enhanced wound healing and neovascularization. This nanocarrier cell surface decoration method is suited for targeted delivery of virtually any type of therapeutic cells and holds promise to be developed as a clinically-relevant cell-based therapy for regenerative medicine and beyond.
Transdermal Deferoxamine Prevents Pressure-Induced Diabetic Ulcers

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Scientific Area: Wound Healing/Fibrosis
Clinical Area: Other

Introduction: The primary reason for compromised diabetic wound healing is impaired neovascularization in response to tissue ischemia. Hypoxia inducible factor-1 alpha (HIF-1α) is the governing transcriptional factor in response to hypoxia and has been shown to be impaired in diabetes. We examined whether activation of HIF-1α via a transdermal drug delivery system (TDDS) of deferoxamine (DFO) was effective to treat and prevent diabetic wounds.

Methods: A TDDS containing the FDA approved small molecule DFO, known to increase HIF-1α activation, was developed. The TDDS was assessed for its physicochemical characteristics and its effects on acute and chronic diabetic wound healing. DFO TDDS application was compared to vehicle patch control in chronic and to 1mM, 100mM drip-on application and vehicle patch control in acute diabetic wounds. Upon closure tensile testing of the wound was performed and histological samples were collected.

Results: The TDDS displayed satisfactory physicochemical characteristics and significantly accelerated excisional wound closure (12 days) vs. 1mM (15.25 days) and 100mM drip-on (15.6 days), and vehicle control patch (19.4 days) (*P < 0.05). No significant differences were observed between the 1mM and 100mM drip concentration. DFO TDDS therapy also reduced chronic diabetic wound healing time by 50% (*P < 0.05). Prophylactic DFO TDDS application was further able to prevent diabetic pressure ulcer formation (*P < 0.05). Histological examination revealed an increase in dermal thickness, collagen density, and vascularity in the DFO patch group (*P < 0.05). Uniaxial skin tensile testing demonstrated increased wound strength in the treatment group according to Young’s modulus (*P < 0.01) and ultimate tensile strength (*P < 0.05).

Conclusion: TDDS application outperforms direct application of DFO solution in diabetic wound healing. DFO TDDS treatment further results in significantly accelerated healing of chronic diabetic ulcers and can be used prophylactically to massively reduce the incidence of ulcer formation in diabetic mice. The developed TDDS improves overall wound quality and can be rapidly translated into clinical practice. Figure Legend (A) An established model of chronic ulcer formation by applying 2 magnets on a dorsal skinfold of diabetic mice was employed. (B,C) Significantly improved ulcer healing was achieved by DFO TDDS treatment. (D) The closed wounds were more vascularized represented by CD31 immunohistochemistry. (E) Picrosirius red staining revealed increased collagen density in the DFO TDDS treatment group.
26.09 - ASC20150013

Delivery of Monocyte Lineage Cells in a Biomimetic Scaffold Enhances Tissue Repair
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Scientific Area: Wound Healing/Fibrosis
Clinical Area: General Surgery

Introduction: Macrophages are thought to play a critical regulatory role in many stages of wound healing, including angiogenesis, reepithelialization, and remodeling. Evidence for the importance of macrophages in these processes comes from experiments demonstrating impaired wound healing in mice following DTR-based ablation of macrophages, genetic knockout of G/M-CSF, or administration of anti-macrophage antiserum. We have previously shown that transplantation of macrophages into excisional wounds on wild type (FVB/NJ) mice significantly increases the rate of wound healing. Here, we expand the analysis to include diabetic wound healing and monocyte transplantation.

Methods: Macrophages derived from the bone marrow of L2G (FVB-Tg(CAG-luc,-GFP)L2G85Chco/J) were seeded on pullulan-collagen hydrogels and transplanted onto splinted excisional wounds on the dorsum of diabetic (FVB.BKS(D)-Leprdb/ChuaJ) mice. Human monocytes isolated from drawn blood were similarly transplanted on pullulan-collagen hydrogels onto splinted excisional wounds on the backs of immunodeficient nude (Foxn1nu) mice. Histologic analysis allowed for in vivo tracking of the survival, localization, and phenotype of transplanted macrophages and monocytes. Microfluidic single-cell gene expression analysis of transplanted L2G macrophages (GFP+Luc+) FACS-isolated on the basis of GFP expression from cutaneous wounds provided further insight into macrophage phenotype and behavior during wound healing.

Results: L2G macrophage-seeded hydrogels improved wound healing compared to un-seeded hydrogel controls on days 4-20 (*p<0.01) in diabetic mice. The average time for complete wound healing was 17.2 days in the macrophage group versus 20.3 days in the control group (*p<0.001). IVIS imaging revealed survival of transplanted macrophages in diabetic wounds through day 20 of wound healing. Microfluidic single-cell gene expression analysis revealed that macrophages transplanted into wounds displayed a predominantly M2 phenotype after being in the wound environment for 24 hours. Human monocyte-seeded hydrogels significantly improved healing compared to un-seeded control hydrogels. The average time to complete healing was 17.8 days in the monocyte group versus 21 days in the control group (*p<0.005). Histologic analysis of monocyte treated wounds showed that transplanted monocytes differentiate in vivo to a predominantly M2 phenotype after 48 hours in the wound environment. Importantly, scar size and quality was not affected in wounds receiving either monocyte or macrophage transplant as compared to controls.

Conclusion: Here we demonstrate that by increasing the number of monocyte lineage cells in the wound site above physiologic levels in diabetic and nude mice the rate of wound healing can be significantly accelerated with no adverse impact on the quality of repair. These findings hold promise for translational medicine aimed at accelerating wound healing across a broad spectrum of diseases.
Heterotopic Ossification is Inhibited in Leptin-deficient (ob/ob) Mice Despite Robust Vessel Growth

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**Scientific Area:** Wound Healing/Fibrosis  
**Clinical Area:** Trauma/Critical Care

**Introduction:** Clinical and laboratory experience has shown that diabetes contributes to delayed wound and bony healing. However, the relationship between diabetes and the generation of heterotopic ossification (HO) following trauma has not been previously studied. By understanding how diabetes potentiates ectopic bone formation, we may be able to identify treatment strategies which target similar local or systemic factors to prevent HO in patients following trauma, large surface-area burns, and surgical procedures.

**Methods:** Male leptin-deficient (ob/ob) or wild type mice (C57BL/6 background) underwent 30% total body surface area burn injury with left hind limb Achille’s tenotomy. At 7 weeks, mice were injected with Microfil contrast and hind limbs were imaged with micro-CT to quantify aggregate vessel volume. Vessel volumes were normalized to hind limb mass to account for differences in limb size between diabetic and wild type mice. A second set of mice underwent micro-CT every 2 weeks up to 9 weeks to quantify HO volume (Hounsfield unit threshold 1000) after adjusting images to remove normal tibia and fibula bone.

**Results:** HO volume in diabetic mice was significantly lower than in wild type mice at 9 weeks following burn and tenotomy injury (3.99 mm\(^3\) v. 6.20 mm\(^3\), p<0.01). Diabetic mice exhibited a decreasing trend in HO volume from week 5 to week 9 based on micro-CT (r=−0.52, p =0.15), suggesting that HO had actually resorbed in these mice. The mean vessel volume in the hind limbs of diabetic mice 7 weeks after burn and tenotomy was significantly greater than the mean vessel volume in wild type mice (20.6 mm\(^3\) v. 4.7 mm\(^3\), p <0.05). When adjusting for limb mass, we found that diabetic legs continued to have more vessel volume (14.1 mm\(^3\)/g v. 7.2 mm\(^3\)/g). Further examination of vessel architecture showed that the diabetic hind limbs had small vessel growth with disorganized character, while the wild type hind limbs had fewer, but larger vessels.

**Conclusion:** Here we use Microfil with micro-CT imaging to compare vessel formation between diabetic (leptin-deficient) and wild-type mice in a model of ectopic bone formation. Our findings demonstrate that despite robust small vessel formation at the site of hind limb trauma, diabetic mice produce less ectopic bone than wild type mice. We also found that diabetic mice actually exhibit resorption of ectopic, suggesting that these mice may be unable to sustain bone growth. This may be related to the disorganized pattern of vessel growth characteristic of the wounds in diabetic mice, as demonstrate by Microfil. Our findings may elucidate additional targets, such as the local vascular milieu, to prevent initial ectopic bone growth or recurrence following surgical excision.
Diabetic Vasculature  Wild type Vasculature

Volume of Ectopic Bone at 9 Weeks

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<th>Volume (mm$^3$)</th>
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<tr>
<td>Diabetes</td>
<td>3.99</td>
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<td>Wild type</td>
<td>6.2</td>
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