points (weeks 1-3) examined (P<0.01). Animals treated with LNT had significant reduction in forepaw diameter, and more importantly, had greater serum anti-OVA antibody levels than controls (P<0.01). However, the potential to mount antibody responses was still lower than sham-operated controls. This finding was corroborated by increased migration of DCs in LNT treated animals as well as the presence of fluorescent bacteria in the transferred LNs, although the total number of both DCs and bacteria were lower than in sham-operated controls. LNT increased LN drainage (P<0.01) and newly formed lymphatic vessels could easily be seen using ICG and NIR. Interestingly, LNT appeared to increase lymphangiogenesis significantly as compared with controls as analyzed by ICG.

CONCLUSION: Our mouse model of non-invasive lymphatic ablation enables us to create a new model of lymphedema. Using this model we have shown that LNT significantly increases lymphangiogenesis, promotes drainage of interstitial fluid, improves antibody responses, and allows for both migration of DCs from the periphery and bacterial presentation in transferred LNs. However, LNT does not completely restore immune responses (at least in the early post-operative period), suggesting that patients who are treated with this approach should continue infectious precautions.

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Short Hairpin RNA Silencing of PHD-2 Improves Neovascularization and Functional Outcomes in Diabetic Wounds and Ischemic Limbs

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PURPOSE: The transcription factor hypoxia-inducible factor 1-alpha (HIF-1a) is responsible for the downstream expression of over 60 genes that regulate cell survival and metabolism in hypoxic conditions as well as those that enhance angiogenesis to alleviate hypoxia. However, under normoxic conditions, HIF-1a is hydroxylated by prolyl hydroxylase 2, and subsequently degraded, with a biological half-life of less than five minutes. The present study investigated the therapeutic potential of inhibiting HIF-1a degradation through short hairpin RNA (shRNA) knockdown of PHD-2 for the treatment of diabetic wounds and ischemic hindlimbs in a mouse model.

METHODS: PHD-2 and non-targeting scramble shRNAs were used to transfect diabetic mouse fibroblasts in vitro. In vivo, ischemic hindlimbs were also treated with PHD-2 shRNA or scramble shRNA. Perfusion was measured with laser doppler,
distal digit tip necrosis was evaluated, surviving muscle bulk was analyzed histologically, and CD31 staining was performed on gastrocnemius muscles. Additionally, 6 mm full thickness wounds were created on the dorsa of diabetic db/db mice. Wounds were injected with either shPHD-2 or shScr. Wound healing was monitored and measured photometrically every two days till closure, and CD-31 staining was performed.

RESULTS: Treatment of diabetic mouse fibroblasts with shPHD-2 in vitro resulted in decreased levels of PHD-2 transcript demonstrated by qRT-PCR and western blot, higher levels of HIF-1α as measured by western blot, and higher expression of the downstream angiogenic genes SDF-1 and VEGFa, as measured by qRT-PCR and western blot. In vivo, shPHD-2 resulted in improved perfusion of ischemic hind limbs compared to shScr, prevention of distal digit tip necrosis, and increased survival of muscle tissue. Delivery of shPHD-2 also accelerated healing of full thickness excisional wounds in diabetic mice compared to shScr control (14.33 ± 0.45 days v. 19 ± 0.33 days), and was associated with an increased vascular density.

CONCLUSION: Knockdown of PHD-2 through shRNA treatment has the potential to stimulate angiogenesis through overexpression of HIF-1α and upregulation of pro-angiogenic genes downstream of HIF-1α, and may represent a viable, non-viral gene therapy for ischemia related applications.

A Transdermal Drug Delivery System for Deferoxamine for the Prevention and Treatment of Diabetic Wounds

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PURPOSE: 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 the response to hypoxia and its function has been shown to be impaired in diabetes. We examined whether upregulation of HIF-1α expression and activity via a transdermal drug delivery system (TDDS) for deferoxamine (DFO) could effectively improve diabetic wound healing and if DFO preconditioning could prevent diabetic ulcer formation.

METHODS: A TDDS containing the FDA approved small molecule DFO, known to enhance HIF-1α transactivity by preventing iron-catalyzed reactive oxygen stress, was developed. The TDDS was assessed for its physico-chemical characteristics, drug release profile and its effects on diabetic wound healing. DFO TDDS application was compared to DFO polymer spray as well as 1mM and 100mM drip-on application. Upon closure, tensile strength testing of the wound was performed and histological samples were collected.

RESULTS: The TDDS displayed satisfactory physico-chemical characteristics and a sustained drug release profile. DFO TDDS application resulted in significantly accelerated diabetic wound closure (12 days) compared to DFO application via polymer spray (14.7 days) as well as 1mM (15.25 days), 100mM drip-on (15.6 days), and vehicle control patch (19.4 days) (*P < 0.05). No significant differences were observed between the polymer spray application and the aqueous solutions. 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). Most interestingly, prophylactic application of the DFO TDDS prevented ulcer formation in a chronic diabetic ulcer model (*P < 0.01).

CONCLUSION: At present, there are no effective therapies to prevent diabetic ulcer formation and only modestly effective technologies to help heal ulcers once they have formed. We have developed a DFO TDDS application that outperforms DFO delivery via polymer spray and aqueous solution in diabetic wound healing with faster closure and improved wound quality. Additionally, the local sustained delivery of DFO via a TDDS represents the first prophylactic pharmacological approach to prevent ulcer formation. As this method involves repurposing a previously FDA approved molecule, it can be rapidly translated into the clinic and ultimately transform the care and prevention of diabetic complications.