and implant exposure. In vivo bacterial luminescence was reduced in the presence of the antibiotic-eluting composite sheet overlay, returning to background signal levels within the study period.

The post-mortem bacterial burden within the peri-implant soft tissue was reduced 600-fold (1.8e2 +/- 1.8e2 versus 1.1e5 +/- 1.5e5, p=0.03), as was average capsule thickness (79 +/- 35 μm versus 274 +/- 194 μm, p=0.001) and relative collagen density within the peri-implant space (31.2 +/- 14.2 % versus 44.5 +/- 15.6 %, p=0.02).

CONCLUSION An antibiotic-eluting nanofiber-hydrogel composite device was designed to reduce the risk of infection and capsule formation following implant-based soft tissue reconstruction. The device inhibited in vivo bacterial growth following implantation of a contaminated implant in a mouse model. Placement of the antibiotic-eluting sheet overlay led to a reduction in soft tissue cellulitis, implant exposure, and peri-implant capsule formation. The technique permits tailoring of mechanical properties and antibiotic release kinetics of the device to suit a variety of surgical applications. The device provides a platform for local delivery of medication into the peri-implant space combined with soft tissue reinforcement.

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Single Locally Implanted Tacrolimus Disk Promotes Long-term Vascularized Composite Allograft Survival via Site Specific Immunosuppression and without Systemic Side Effects

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PURPOSE: More than 185,000 amputations occur in United States each year. The most common is a partial hand amputation (61,000), and the majority (77%) of cases are due to traumatic accidents. Vascularized composite allotransplantation (VCA), in the form of hand allotransplantation, provides another option for hand reconstruction. The skin component of VCA is highly antigenic and mandates daily intake of systemic immunosuppressive drugs. Currently tacrolimus (TAC) and mycophenolic acid (MPA) are the two primary immunosuppressive drugs used in transplant patients. These drugs have narrow therapeutic window, significant pharmacokinetic variability, non-adherence to these drugs, putting the patients at risk for rejection or toxicity. These drug related complications compromise the long-term outcomes. We prepared a novel, re-loadable drug delivery system that consists of an encapsulated sustained-release version of oral TAC alone or combined with other drugs that provides sustained drug release into the graft tissues and regional lymph nodes, while minimizing systemic blood levels. This results in lower overall systemic drug exposure, while the sustained loco-regional delivery facilitates long-term VCA survival.

METHODS: TAC loaded polycaprolactone disk were prepared by solvent casting. Following orthotopic hind limb allotransplantation, animals (n=6/group) received no treatment (Group 1), TAC 1mg/kg/day intraperitoneally (Group 2), or one TAC disk in the transplanted limb (Group 3) or in the contralateral un-transplanted limb (Group 4). TAC levels in blood and tissues were measured using LC-MS/MS. In addition to allograft survival, systemic toxicity was evaluated using metrics such as % change in body weight (BW), blood glucose, and creatinine clearance (CrCl).

RESULTS: A single TAC disk (5mg, 5 % w/w) resulted in blood levels between 2 to 5 ng/ml for nearly 100 days. High levels of TAC were achieved locally in the transplanted limbs, when compared to levels in the contralateral limbs (**p<0.001). These levels could inhibit immune activation and sustained allografts survival for >150 day (Group 3). While animals received no treatment or TAC disks in the un-transplanted limbs (Group 1 and 4) had median survival 8 ± 4 days and 71 ± 7 days. Long term allograft exposure to locally delivered TAC induced donor specific hypo-responsiveness. Lower levels of IFNα+ cytotoxic T cells, while higher levels of IL-10+ T regulatory cells were observed in draining lymph nodes isolated from transplanted limbs. This could suggest the mechanism behind long term survival by locally delivered TAC. No signs of systemic toxicity were observed in animals received TAC disks, as compared to animals received standard systemic immunotherapy.

CONCLUSION: A single TAC disk implanted into the transplanted limb was effective in sustaining allograft survival
via loco-regional immunosuppression, without systemic side effects. Therapeutic modulation of the loco-regional immunity may influence the outcome of VCA and underline that tissue-specific immune-response warrants further investigation in VCA. Our study offers an alternative to the current treatment paradigms which use systemic immunosuppression, to one of loco-regional immunosuppression using locally implantable biomaterials. With this research, we hope to establish the basis for the development of more advanced technologies for targeted immunosuppressive drug therapy.

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Jun Drives A Fibrogenic Cutaneous Wound Healing Response Through Differential Action On Distinct Fibroblast Subpopulations

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PURPOSE: Skin fibrosis and scarring can result in devastating disfigurement and permanent functional loss. Currently, there are no treatment modalities able to prevent or reverse this fibrotic process, and scars and their consequences result in an enormous medico-economic burden. Thus, it is of paramount importance to better understand the key pathogenic mechanisms driving this pathological fibrotic process. We recently described a mouse model in which overexpression of JUN, an AP-1 transcription factor, can be induced to produce global tissue fibrosis. Here, we explored the effects of JUN overexpression in skin scarring and fibrosis and the ability of our mouse to model hypertrophic scarring in response to wounding.

METHODS: Stented excisional dorsal wounds were created in transgenic JUN (c-Jun<sup>cre</sup>) R26-M2rtTA) and Rosa (Rosa26-rtTA) control mice. Doxycycline (2mg/ml) was used to induce JUN overexpression in the wound beds on the day of surgery (POD 0) and every other day until the wounds healed (postoperative day 14, POD 14). On alternate days throughout the healing response, wounds were harvested and analyzed histologically for thickness and collagen deposition, and by fluorescent activated cell sorting (FACS) to compare the relative percentages of fibroblast subpopulations. To study the role of JUN in human scars, dermal fibroblasts were isolated from hypertrophic scars (HTS) and healthy control skin (NS) and transduced to knock out JUN using a CRISPR-Cas 9 method. Proliferation and apoptosis were compared in the knock-out (KO) and non-KO human dermal fibroblasts.

RESULTS: The wounds of JUN mice healed at a significantly accelerated rate between POD5 and POD14 (*p<0.05). Compared to the wounds of Rosa control mice, the scars of JUN mice on POD14 were significantly thicker, and although collagen content was not different, it was more disordered on Hematoxylin and Eosin staining, and brighter and more branched upon computational assessment of Picrosirius stained wounds (*p<0.05). JUN overexpression resulted in a significant expansion of reticular fibroblasts at the expense of lipofibroblasts, evident on POD 7 (*p<0.05). Translating these results to human scars; JUN CRISPR-Cas 9 deletion increased apoptosis and decreased proliferation of primary cultures of HTS and NS fibroblasts.

CONCLUSION: JUN overexpression increases the fibrotic cutaneous wound healing response by significantly expanding reticular dermal fibroblasts at the expense of the dermal lipofibroblasts. In addition, assessment of HTS and NS fibroblasts isolated from human skin indicate that JUN also mediates the fibrotic response in human disease by inhibiting apoptosis and driving proliferation of the dermal fibroblast subsets. Thus, our novel inducible JUN mouse model can be used to explore the mechanisms driving HTS and other pathological skin fibrosis and facilitate targeted identification of new treatments.

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Altered Iron-Mediator Expression Identifies Chemotherapeutic Effects of Deferoxamine on Head and Neck Squamous Cell Carcinoma

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