METHODS: Male C57BL/6 mice were subjected to a musculoskeletal extremity trauma model of HO formation involving a 30% total body surface area dorsal burn and Achilles tenotomy. Single cell RNA sequencing (10X genomics) and downstream unsupervised clustering analyses were performed on the extremity injury HO site at baseline (D0), during inflammation (D3), and mesenchymal progenitor cell condensation (D7) (n=3/group).

RESULTS: Canonical correlation analysis yielded 14 transcriptionally unique cell clusters identifiable at the injury site with characteristic profiles attributable to phenotypically distinct cell types. While recruited granulocytes significantly reduced in numbers from 36.1% on day 3 to only 10.7% at day 7, macrophages/monocytes and dendritic cells were the predominant cell populations and constituted over 35% of total cells on day 7. Subpopulation analysis revealed distinct monocyte/macrophage clusters with M2 characteristic gene expression including Mrc1 (CD206), H2-Eb1 (MHC II) and Cd163 as well as Arg1 (cluster 1, 3, 5 and 8). While cluster 1 showed expression of all three markers, cluster 3 showed high expression of Arg1 but not Cd163 indicating that these are phenotypically unique cell populations with distinct functions. We further observed a significant increase in HO progenitor subpopulations (cluster 2, 4, and 6) on day 7 which were almost entirely absent on day 3. Clusters 2, 4, 6 and 11 showed high expression of Pdgfra, a marker known to identify mesenchymal progenitor cells. Cluster 11 completely disappeared on day 7, suggesting that these cells likely differentiated into HO forming cells. Interestingly, mesenchymal cell clusters further demonstrated high expression of chondrogenic differentiation genes Acan and Sox9 as early as on day 3 indicating early cell fate determination.

CONCLUSION: To elucidate HO pathophysiology, it is critical to characterize the intricate interactions of inflammatory cells and progenitor cells. Using single cell RNA sequencing as a novel tool, we identify the presence of yet unidentified and functionally distinct subpopulations of monocytes and progenitor cells with unique transcriptional characteristics. Isolation of cells based on these findings may allow for a more granular understanding of HO and future design of targeted treatment.

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Minimizing Implant Infection and Capsular Contracture Through the Use of Antibiotic-Eluting Nanofiber Coatings

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PURPOSE: Bacterial contamination following implant-based soft tissue reconstruction contributes to significant healthcare costs and patient morbidity. In addition to acute infection, subclinical bacterial colonization is thought to contribute to long-term capsular contracture. The purpose of this study was to design an antibiotic-eluting nanofiber-hydrogel composite sheet for use in implant soft tissue pocket reinforcement. A murine implant infection model was developed to test the impact of the device on implant infection and infection-associated capsule formation.

METHODS: Polycaprolactone (PCL) impregnated with linezolid and rifampicin was electrospun into a random-pattern sheet and suspended within a nanofiber-hydrogel composite. Interfacial bonding between the nanofibers and hydrogel matrix was used to improve structural integrity of the material. Mechanical properties and antibiotic release kinetics were assessed in vitro. Silicone disk implants were incubated with a bioluminescent Staphylococcus aureus strain for 24 hours. Biofilm formation was confirmed via crystal violet staining. Thirty-five mice were implanted with either the infected implant alone, infected implant with overlying composite sheet, or infected implant with overlying antibiotic-eluting composite sheet. Bioluminescence imaging was used to assess the in vivo bacterial burden between postoperative days 0 and 15. Postmortem bacterial colony forming unit (CFU) quantification, histology, and immunohistochemistry were performed on harvested tissues.

RESULTS: A 1mm-thick nanofiber-hydrogel composite sheet embedded with linezolid and rifampicin was designed and demonstrated favorable mechanical properties, suture-ability, and antibiotic release kinetics. Use of the antibiotic-eluting composite sheet device resulted in complete prevention of clinical signs of cellulitis.
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 IFNγ+ 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