fat formation. Thirty percent of CD patients develop strictures, eighty percent of which will require surgery. Creeping fat is associated with stricture formation, but its role in intestinal fibrosis remains unclear. Here, we present a novel surgical model of intestinal fibrosis and show by lineage tracing that creeping fat adipocytes convert to fibroblasts that contribute to fibrosis.

**Methods:** We developed a novel surgical model of IBD that avoids the chronic use of caustic agents by creating a longitudinal, anti-mesenteric colotomy in the mouse transverse colon that is closed transversally. We performed Masson’s trichrome staining to assess collagen deposition. Finally, we performed lineage tracing of mature adipocytes in Adiponectin-Cre; mTmG mice and characterized adipocyte-derived cells by immunostaining.

**Results:** Our surgical model mimics key features of human strictures, including the formation of creeping fat around the injury site, increased bowel wall thickness, collagen deposition, and transmural adipocyte infiltration by post-operative day (POD) 7. Immunostaining for adipocyte and fibroblast markers confirmed the presence of transmural adipocytes adjacent to fibroblasts. Lineage tracing of mature adipocytes in Adiponectin-Cre; mTmG mice revealed adipocyte-derived cells that infiltrate the wound site that lose expression of adipocyte lineage markers, gain expression of fibroblast markers, produce collagen, and respond to TGF-β signaling.

**Conclusions:** Our novel surgical colotomy model represents a viable approach to study intestinal fibrosis and creeping fat without the long-term use of caustic agents. Lineage tracing of mature creeping fat adipocytes demonstrates that adipocytes convert to fibroblasts that infiltrate the injury site and participate in fibrotic responses. Taken together, these findings suggest that creeping fat contributes to intestinal fibrosis in part through the local conversion of adipocytes to fibroblasts.

**Purpose:** While tissue engineering offers the promise of revolutionary innovation, scalable three-dimensional tissue culture is limited by the diffusion of nutrients and oxygen making media perfusion obligatory. Unfortunately, the cost of bioreactors for large construct tissue culture can be prohibitive, with a typical perfusion chamber costing several thousand dollars, and even small petri-dish-sized devices costing hundreds of dollars each. We have developed a low-cost perfusion setup that seals collagen-based perfusable cellular constructs within a sterile PDMS well between coverslips, allowing for repeated live-imaging of perfused 3D engineered tissues. Herein we describe fabrication of this novel system and validate its utility.

**Methods:** Molds and frames were designed on 3D-modeling software (Fusion 360) and printed on a Prusa i3 MK3S 3D printer in poly(lactic acid) (PLA). Molds were filled with poly(dimethyl siloxane) (PDMS), which was cured to form chambers, bubble traps, mason jar lid chambers, and media reservoir lid adapters. In total, the tissue culture chamber device, mason jar lid inset, media reservoir lid, and bubble trap require 4, 1, 2, and 4 unique printed components, respectively.

**Results:** Each perfusion chamber can be assembled for under 8 USD per device and reused repeatedly. The current model has a tissue chamber custom-built with 18x10x4 (LxWxH)mm cubed dimensions, but this chamber can be readily customized to experiment-specific dimensions. These devices allow cellular hydrogel constructs to be maintained in a sterile environment after assembly, perfused at varying rates to expose cells to different levels of shear stress, and the cells can be intermittently imaged with light, fluorescent or confocal microscopy - an unparalleled benefit for monitoring of experiments and collection of timepoint imaging data. The perfusion circuit consists of autoclavable glass and PDMS components, including a bubble trap, a crucial component of the circuit for preventing air bubbles that can damage cells and block microchannels, and a lid adapter, which allows 50 mL conical tubes to serve as self-oxygenating media reservoirs. Media changes can be performed via peristaltic pump perfusion or with syringe-based cell culture techniques for static culture. Constructs have been perfused within standard incubators for up to 14 days demonstrating normal cell viability without contamination or evidence of infection, with longer perfusion culture intervals (>1 month) currently being tested.

**Conclusion:** The increasing accessibility of 3D-modeling and 3D-printing has enabled rapid prototyping of devices to address the problems that we face as surgeon-scientists.
We have developed a low-cost tissue-engineering perfusion circuit that facilitates 3D-tissue-culture while allowing for repeated live-imaging as the cultured tissue develops. The instructions for our setup can be utilized to replicate our devices in other labs, and these designs can be readily customized to meet the needs of specific experimental aims.

**QS3**

**CRISPR/Cas9 Editing of Autologous Dendritic Cells to Enhance Angiogenesis and Wound Healing**

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**Purpose:** Dendritic cells (DCs) are a heterogeneous cell population which critically regulates the adaptive immune response. Depending on their activation status, DCs can also promote peripheral immune tolerance, thus limiting the activation of the immune system and tissue damage. The N-myct downregulated gene 2 (Ndrg2) is highly expressed in DCs and limits the secretion of vascular endothelial growth factor (VEGF), which is critical for wound healing. Cell based therapy approaches using DCs have been approved by the FDA and clinical trials using DC immunotherapy are being performed against a variety of cancer types. However, the role of DC therapy for wound healing has not yet been investigated.

**Methods:** Hematopoietic progenitor cells were isolated from the bone marrow of mice and differentiated into DCs over a 7-day in vitro culture period. Pharmacologic down-regulation of Ndrg2 was performed by treatment of cultures with 1,25-dihydroxyvitamin D3 (VD3) and the angiogenic potential of the treated cells was evaluated by endothelial cell (EC) tube formation assays. Cytokine secretion of DCs was measured in the conditioned media using Luminex multiplex assays. To permanently knock out Ndrg2 in DCs, a CRISPR/Cas9 gene editing approach was developed, using Cas9/sgRNA-ribonucleoproteins and electroporation. The determine the impact of genetically edited DCs on wound healing, splinted excisional wounds in C57BL6/J (wildtype) mice were treated weekly with pullulan-collagen hydrogels seeded with Ndrg2-knockout (KO) DCs, control DCs which had undergone electroporation only, or blank hydrogels. The transcriptomic impact of Ndrg2 downregulation on DC fate was evaluated by microfluidic single-cell RNA sequencing (scRNA seq) of Ndrg2-KO DCs, VD3-treated DCs and control DCs.

**Results:** Ndrg2 down-regulation lead to a significantly stronger EC tube formation in co-cultures with VD3-treated DCs, and strongly enhanced VEGF secretion compared to untreated DCs in vitro. A CRISPR/Cas9 editing pipeline was developed for KO of Ndrg2 in DCs with a transfection rate and editing efficiency of > 90% shown by Sanger Sequencing. Excisional wounds treated with Ndrg2-KO DCs demonstrated significantly accelerated healing compared to control DCs and blank hydrogels. scRNA seq revealed that Ndrg2 downregulation strongly induced Vegfa expression and anti-oxidant transcriptomic signatures.

**Conclusion:** Our data indicate that KO of Ndrg2 in DCs strongly enhances their secretion of VEGF, thus promoting angiogenesis and accelerating wound healing. Given the ready availability of DCs from the human blood through established leukapheresis protocols and easy multiplication in vitro, CRISPR/Cas9 editing of DCs is a promising new approach to induce wound healing and soft-tissue regeneration.

**QS4**

**In Vivo Quantitative Analysis of Subcutaneous Membranous Layers -Superficial And Deep Fascia- In Eleven Regions of the Human Body**

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