wounds (1 ± 0.13). Similarly, RT-qPCR data revealed that relative mRNA levels of SMAD3 were significantly lower in fibronectin-treated wounds (0.34 ± 0.11) than in control wounds (1 ± 0.43). Lastly, protein level correlation with ELISA identified significantly lower TGF-β1 concentrations in fibronectin-treated wounds (2682 ± 515.83 pg/mL) compared to control wounds (5244.5 ± 700.08 pg/mL).

**Conclusion:** Hydrogel-facilitated delivery of fibronectin significantly improved the rate and quality of wound healing in a porcine chronic irradiation wound model. Thus, this novel mechanism of fibronectin supplementation demonstrates potential for treating these otherwise nonhealing wounds.

6

**Biomimetic Microtissue Keloid Scar System Using Keloid-derived Fibroblasts and Macrophages**

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**Purpose:** Keloid is a disease that affects millions of patients and has relatively few effective treatment options. Unfortunately, traditional 2D monolayer culture of keloid derived fibroblasts show little resemblance to the pathological process in vivo. Additionally, keloid is notably a pathology largely specific to humans, without good in vivo models available. To fill this gap, we have developed a 3D in vitro microtissue keloid scar model with human keloid derived fibroblasts and peripheral blood derived macrophages.

**Methods:** Under IRB approval, keloid tissue was from patients and used to develop a human keloid derived fibroblast line. Keloid spheroids were fabricated from these keloid derived fibroblasts and human peripheral blood derived macrophages. Commercial human skin-derived fibroblast and 2D monolayers were used as controls. Quantitative PCR with fibrosis genes (collagen-1, αSMA, TNF, IL1β, IL6 and TGFβ) and immunofluorescent staining with (collagen-1, αSMA, CD68 and pSTAT3 were performed to validate the keloid spheroids as an effective keloid model in vitro. In addition, we performed qPCR fibrosis microarray for fibrosis-specific gene expression. Lastly, the Affymetrix PrimeView array was used to evaluate genome-wide comprehensive gene expression to assess whether the keloid spheroid mimicking behavior of keloid tissue regarding gene expression level.

**Results:** Spheroids had significantly higher expression levels of all fibrosis related genes compared to the 2D monolayer control. Among the spheroid groups, keloid spheroids had much higher gene expression levels of collagen-1 and αSMA, which was confirmed by the immunofluorescent staining with the same correspondence proteins. Interestingly, keloid spheroids showed lower gene expression levels of common fibrosis related cytokines (TNF, IL1β, IL6 and TGFβ). However, IF of pSTAT3 was upregulated in keloid spheroid, which is consistent with previous literature of keloid research. Lastly, qPCR fibrosis array and human comprehensive gene expression assay validated the result of qPCR and indicated that macrophages in the keloid spheroids showed signs of polarization in both M1 and M2 directions.

**Conclusions:** We have developed a keloid mimicking spheroid microtissue as a more physiologically relevant in vitro keloid model for drug development and research exploring gene and protein expression pathways. This platform recapitulates important features of keloid behavior not seen in 2D culture. Future work will include screening of keloid spheroid responses to potential therapeutic treatments.

**QUICK SHOTS**

**QS1**

_Creeping Fat Adipocytes Drive Intestinal Fibrosis Through Adipocyte-to-fibroblast Conversion in a Novel Model of Inflammatory Bowel Disease_

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**Purpose:** Crohn’s disease (CD) is a subtype of inflammatory bowel disease (IBD) characterized by patchy, transmural inflammation throughout the digestive tract and creeping
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 cover slips, 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) mm³ 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.