KEYSTONE SYMPOSIA

Fibrosis and Tissue Repair 2022

Describing the Progression of Bleomycin-Induced Fibrosis in a Mouse-Lung Model via Oropharyngeal Administration

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Introduction

Bleomycin (BLM) is commonly used for the induction of fibrosis in various mouse organ models, including Non-Alcoholic Steatohepatitis (NASH) liver models and Idiopathic Pulmonary Fibrosis (IPF) liver models. However, fibrosis induction by BLM for IPF lung models has not been well quantified nor characterized. Here, FibroNestTM, a novel, cloud-based, and quantitative image analysis platform, is used to analyze fibrosis progression in BLM-induced mouse lung fibrosis by generating and measuring quantifiable Fibrosis Traits (qFTs).

Methods

- Control-PBS group (d21, n=6)
- 3 BLM groups (d7, n=8 | d21, n=6 | d35=5)
- BLM exposure was ended after d21.
- FFPE histological sections of both lungs were stained (Masson Trichrome) and scanned at 20X with an Aperio AT2 Scanner
- FibroNest™ measured and quantified the fibrosis phenotype for its collagen content and structure (12 traits), the morphometric traits of the collagen fibers (13 traits), and fibrosis architecture traits (7). Each trait is described by a histogram from which 7 statistical measurements are derived.
- Aggregation of the principal qFP in one or multiple composite scores
- Principle qFTs are combined into several normalized composite scores including the overall Phenotypic Composite Fibrosis Score (Ph-CFS) and the Architecture Composite Score.
- Heat Chart show the relative change of the principal qFPs

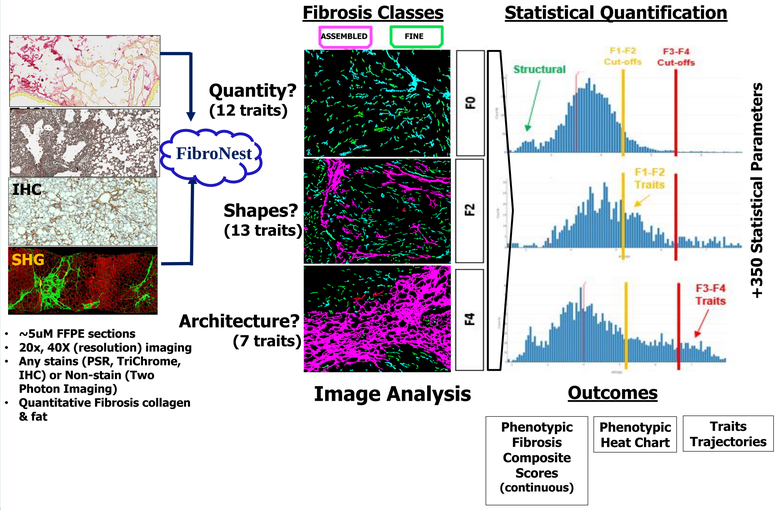
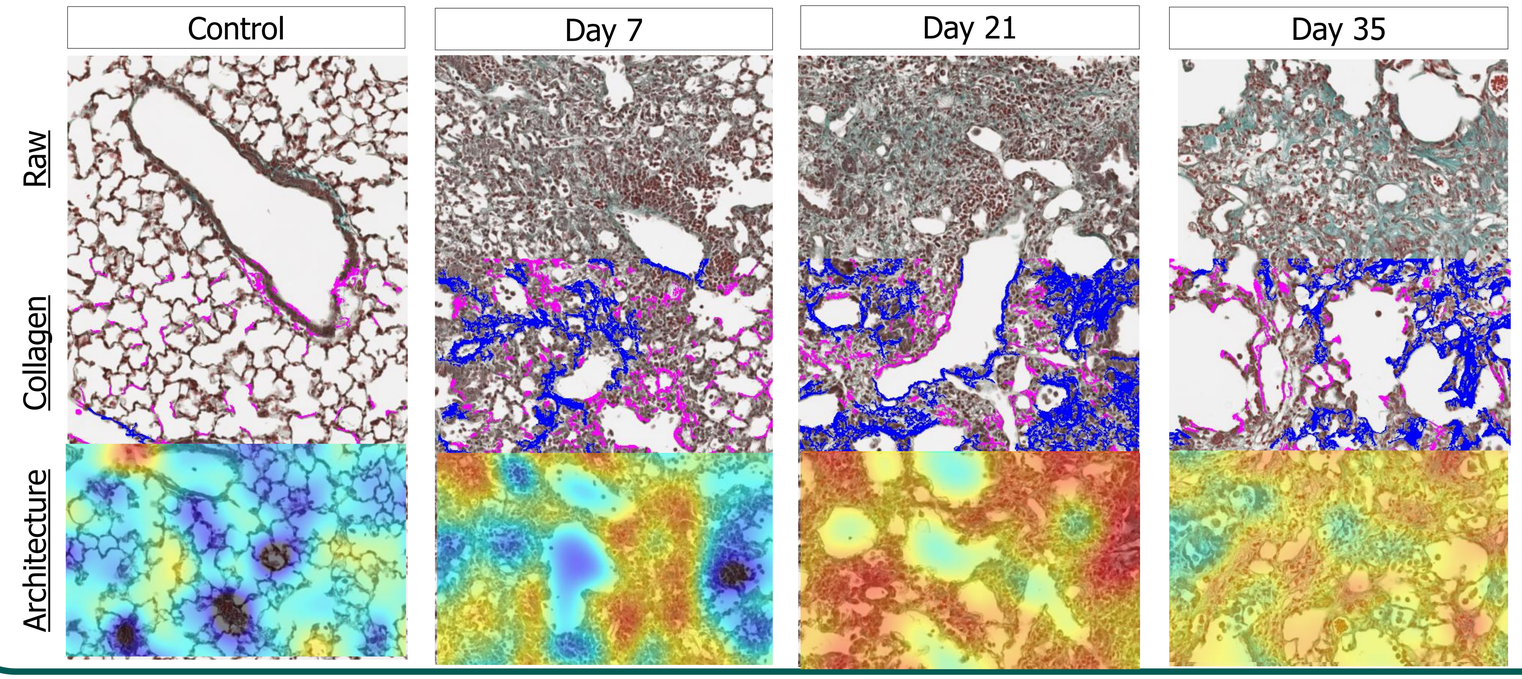


Figure 1: FibroNest™ workflow illustrated (image upload). FibroNest™ quantifies fibrosis at 3 levels, including collagen content, fibers morphometry and collagen texture.

Results: Representative Images



Results: Phenotypic Maps

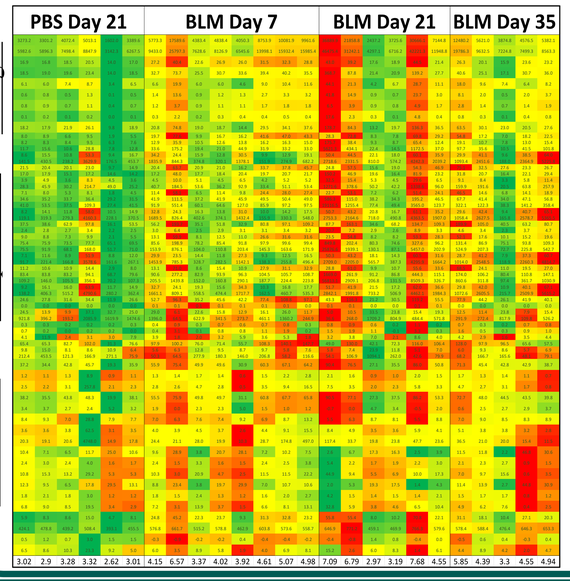


Figure 2: For each patient (column) the Fibrosis Phenotypic Maps (Left) visualizes the relative severity (green to red) of the quantitative fibrosis traits (qFTs) as quantified from the image, and automatically selected to account for variability between groups. The normalized quantitative traits values are combined to generate a phenotypic Fibrosis composite score. The similar concept can be used for Steatosis phenotype (including % steatosis, and mean, median, standard distribution, skewness and kurtosis fat vacuole size distribution, illustrated below).

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Results: Composite Scores

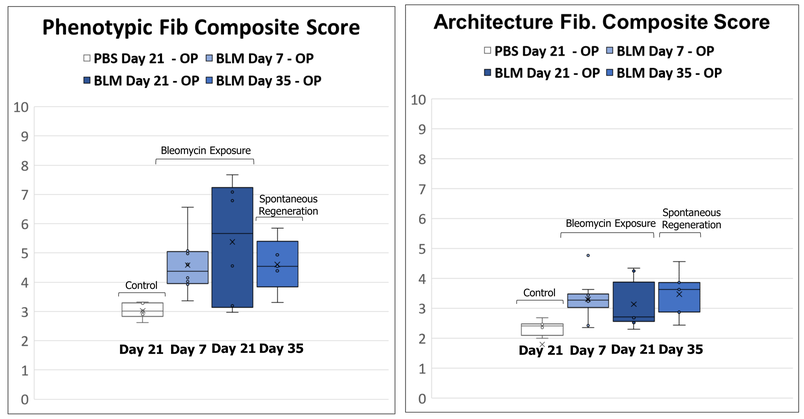

Figure 4. The Phenotypic Fibrosis Composite Scores (Ph-FCS) describe the variability of the severity of fibrosis from BLM exposure at each sacrifice date. The BLM-response was aggressive and was especially varied at day 35. The Architecture Fibrosis Score is a component of the Ph-FCS which accounts for the macro-organization of fibers. It shows a softer response to BLM, suggesting that it is less responsive to collagen amount (instead impacted by collagen organization.

Conclusions

Quantitative Digital Pathology of stained lung sections was able to quantify the progression and severity of fibrosis in OP-BLM mice. This model was found to have rapid and aggressive proliferation of fibrosis up to day 21. The variability generated by this method at d21 disappears at d35, perhaps due to dose saturation. Architecture composite scores maintained a gentler progression of fibrosis phenotypes. This indicates that the architecture score is less sensitive to the quantity of fibrosis compared to how the fibrosis itself is organized.