KEYSTONE SYMPOSIA

TISSUE FIBROSIS AND REPAIR

Advanced Skin Image Analysis for Evaluation of Bleomycin-induced Skin Fibrosis in Mouse Scleroderma Model

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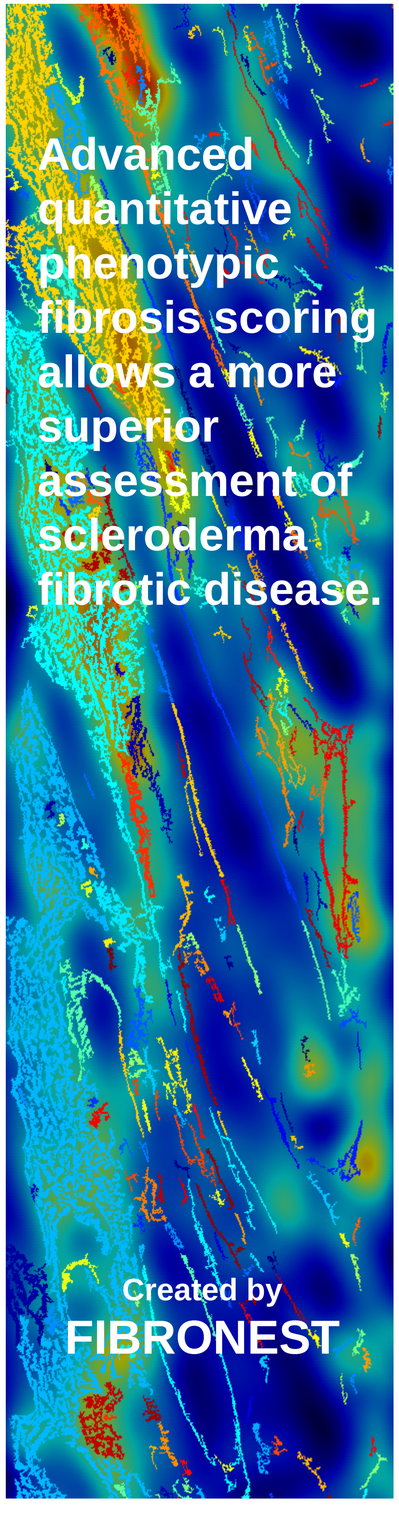


1 Introduction

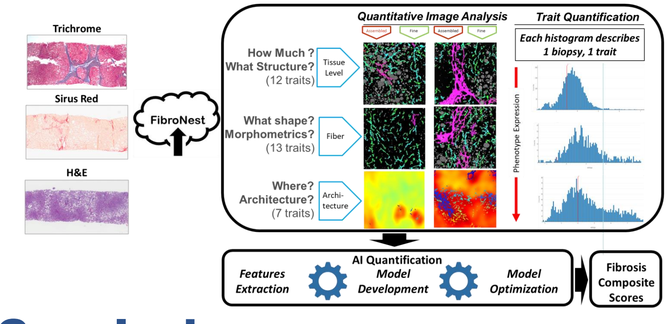
Scleroderma is a chronic autoimmune disease characterized by inflammation, vasculopathy, and fibrosis of the skin and internal organs. Extraneous collagen deposition causes hardening, tightening, and thickening of the skin. Here, we use a mouse model of scleroderma to mirror the pathogenesis of this human skin fibrosis disease. The current standard method of determining collagen level in skin is using hydroxyproline assay which can be cumbersome with low dynamic range and only yield a basic measurement of collagen amount.

2 Aim

In this study, we use FibroNest™, a cloud-based quantitative image analysis platform, to provide advanced quantitation of skin fibrosis that generates a continuous phenotypic scoring from histological skin images in bleomycin- induced skin fibrosis mouse model.

3 Method

- Mice were given 3x/ week subcutaneous injection of bleomycin (0.1unit/ mouse) to induce dermal fibrosis.
- Skin sections were obtained from animals treated with bleomycin (BLM, n=8/ grp) using time course at 7, 21, and 35 days or with PBS control (n=5/ grp).
- *An additional group was treated with BLM until day 35 and sacrificed at day 64 to study potential regression.*
- Histology slides were stained with Masson's trichrome for collagens and imaged 20X with an Aperio AT2 Digital Pathology System. Skin image analysis concentrates on the dermis region of the skin and excludes the hair follicles, glands, and other structures.
- FibroNest™, a cloud-based quantitative, single fiber, image analysis platform, was used to quantify the fibrosis phenotype including 32 traits for Collagen content, fiber Morphometry, and Architecture. Principal quantitative fibrosis traits (up to 315 qFTs) are consolidated to generate a normalized Phenotypic Composite Fibrosis Score (Ph-FCS).



4 Results

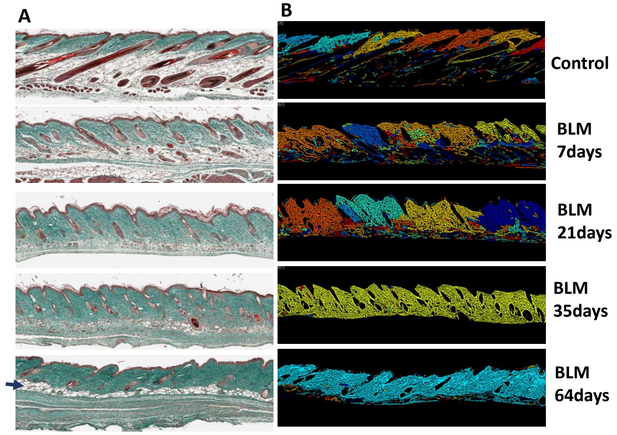


Figure 1. A. *Skin histology from bleomycin-treated mice or control stained with trichrome (green) for collagen. Lost of fat layer as fibrosis (collagen) develops (BLM 7 to 35days) and re-emergence of fat (arrow) during regression (at day 64).*
B. *FibroNest image analysis. Collagen fibers displayed in colors. Each color shows an "individual" fiber. As fibrosis progress, old and new fibers coalesce and became a more complex larger fiber. Note the single color in days 35 and 64.*

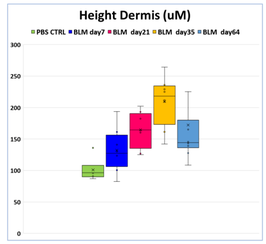


Figure 4. Skin Dermal Thickness. *Bleomycin increases thickness of the dermis from day 0 to 35. Dermal thickness regresses at day 64 (with bleomycin treatment stop at day 35).*

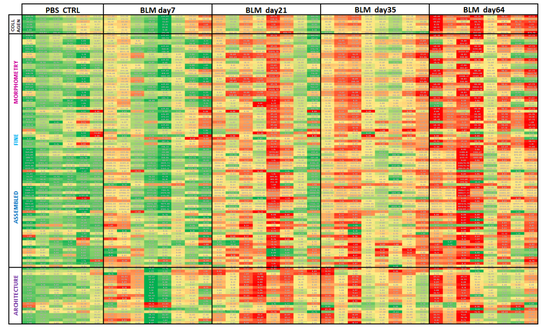


Figure 2. Heatmap of the phenotypic fibrosis quantification. *Principal fibrosis parameters (row) are normalized, and their progression is showed in color scale chart. Each column represents an animal. Fibrosis parameters are combined to form the phenotypic Fibrosis Composite Scores (Ph-FCS) (Figure 3A).*

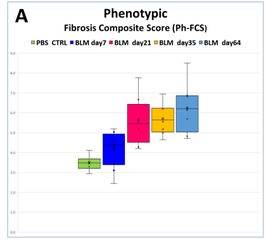
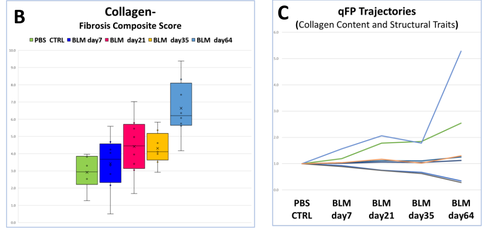
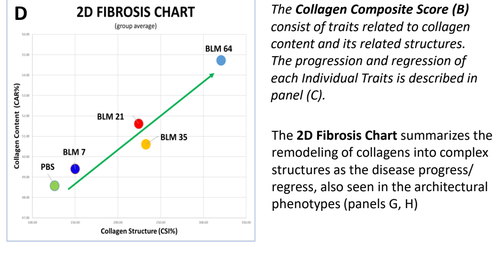


Figure 3(A-H). A. The FibroNest's Fibrosis Phenotypic Score*. Ph-FCS is continuous and incorporates the three phenotypic components: the* ***Collagen content*** *levels and related structures, the* ***Single fiber morphometry****, and the* ***Fibrosis Architecture*** *and their related changes (remodeling).*

Collagen Content and Structure

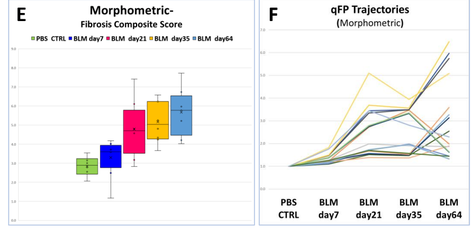




The ***Collagen Composite Score (B)*** *consist of traits related to collagen content and its related structures. The progression and regression of each Individual Traits is described in panel (C).*

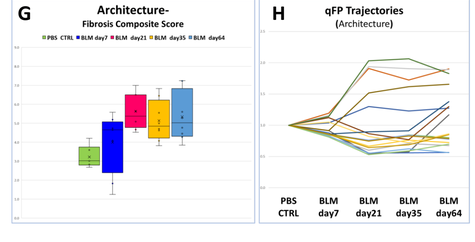
The **2D Fibrosis Chart** summarizes the remodeling of collagens into complex structures as the disease progress/ regress, also seen in the architectural phenotypes (panels G, H)

Single Collagen Fiber Morphometry



Morphometric scores and Trajectories E,F describe the signatures of progression and regression of fiber morphometry, as they grow from Fine to Assembled complex fibers.

Fibrosis Architecture



Architectural phenotype, specifically the built up (and regression) of complex fiber structures are quantified in G,H.

5 Conclusions

- Bleomycin-induced skin fibrosis shows a time dependent increase in dermal thickness, disappearance of fat layer, and increases in fibrosis composite scores including Phenotypic, Collagen content, Morphometric, and Architecture. At day 64, regression (due to stop in bleomycin treatment at day 35) was seen for dermal thickness but not in the fibrosis scores.
- Advanced fibrosis analysis of the skin images with FibroNest™ provides detailed phenotypic information to evaluate fibrosis severity and progression. Collagen content, morphometric and architecture scores help characterize fibrosis beyond a simple collagen measurement of the skin. This can be of clinical importance in guiding therapeutic strategies in treating skin fibrosis in scleroderma disease.

6 Contact information

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