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

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Introduction
Scleroderma is a chronic autoimmune disease characterized by inflammation, vasculopathy, and fibrosis of the skin and internal organs. Exogenous 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.

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

Method
- Mice were given 3x/week subcutaneous injection of bleomycin (0.1 unit/mouse) to induce dermal fibrosis.
- Skin sections were obtained from animals treated with bleomycin (BLM, n=8/group) using time course at days 7, 21, and 35 days or with PBS control (n=5/group).
- 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 using an Aperio AT2 Digital Pathology System. Skin image analysis concentrates on the dermal region of the skin and excludes the hair follicles, glands, and other structures.
- FibroNestTM, 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 df's) are consolidated to generate a normalized Phenotypic Composite Fibrosis Score (Ph-FCS).

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 FibroNestTM provides detailed phenotypic information to evaluate fibrosis severity and progression. Collagen content, morphometric and architectural 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.

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Results

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

Figure 2. Heatmap of the phenotypic fibrosis quantification. Principal fibrosis parameters (rows) 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 and B). 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).

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 5. (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).