KEYSTONE SYMPOSIA
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Single-fiber Digital Pathology Image Analysis accurately quantifies the severity of the fibrosis phenotype in an experimental rodent model of systemic sclerosis

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BACKGROUND

Systemic sclerosis (SSc) is a severe autoimmune disease characterized by an immune dysregulation, vasculopathy, and fibrosis of the skin and internal organs. The development of efficient anti-fibrotic therapeutic remains an important challenge. Preclinical studies based on animal models of SSc have allowed several advances concerning the mechanisms of fibrosis.
However, conventional analysis in preclinical study, such histological staining, though considered as gold standard methods, mainly allow semi-quantitative, low-sensitive and analysis observer-dependent of fibrosis development and progression.

AIM

To describe the severity and changes of the histological phenotype of skin fibrosis in an experimental model of SSc, by a novel Phenotypic Fibrosis Composite Score (Ph-FCS) derived by single-fiber and quantitative digital image analysis combined with Artificial Intelligence (AI)

MATERIALS AND METHODS

- **Hypochlorous acid (HOCl)** solution → 9.6% NaClO to 100 mM KH_2PO_4 (pH 6.2), in a 1:60 ratio
- Administration: daily intradermal injection (300 µL) into the shaved back of mice for 5 days per week, for a cumulative of 6 weeks (Fig. 1A). Control Group (N=11) and HOCl Group (N=12)
- Skin histological sections were stained with H&E (for skin architecture, inflammatory cell infiltrate and dermal thickness quantification) and Masson's trichrome (for collagen)
- Stained skin sections were imaged at 20X magnification on a slide scanner Axioscan Z1 (Fig. 1-B)
- **Single-fiber Digital Pathology Image Analysis**, fibrosis phenotype was quantified by FibroNest (PharmaNest Inc, Princeton, USA), a cloud-based quantitative, single fiber, image analysis platform. Analysis included 32 traits for collagen content and structure, fiber morphometry, and fibrosis architecture (measures the organization of the fibers) (flow chart below), generating 315 quantitative Fibrosis Traits (qFT). Principal traits are identified and assembled into continuous scores for each phenotypic dimension (Fig. 3 c-d-e). The phenotypic Fibrosis Composite scores recapitulates all the dimensions into one scores (Fig. 3-i)

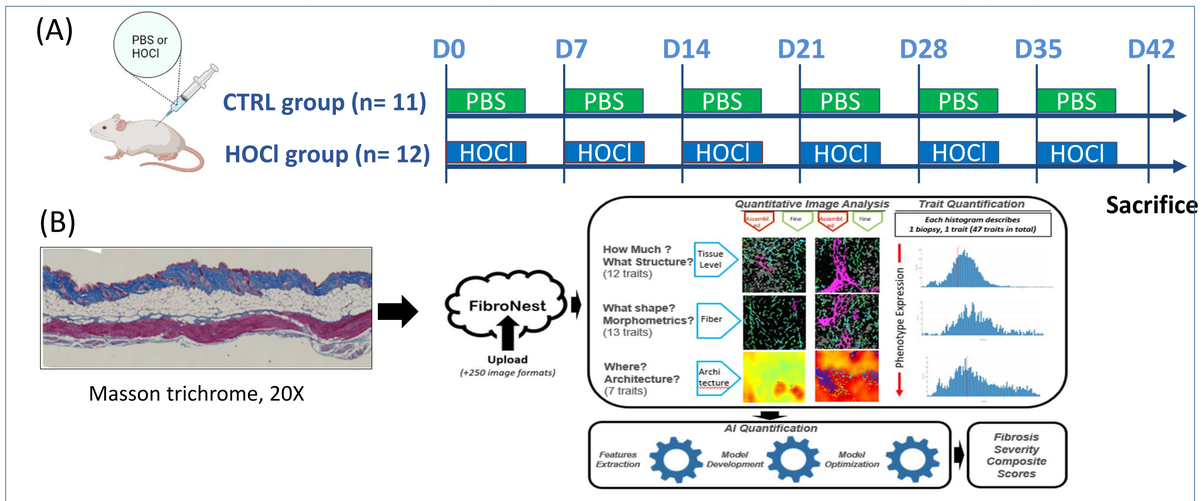
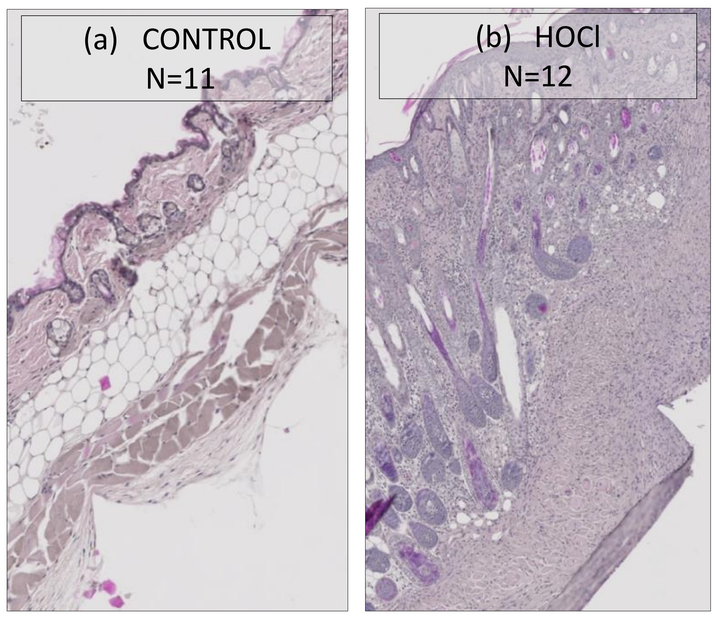
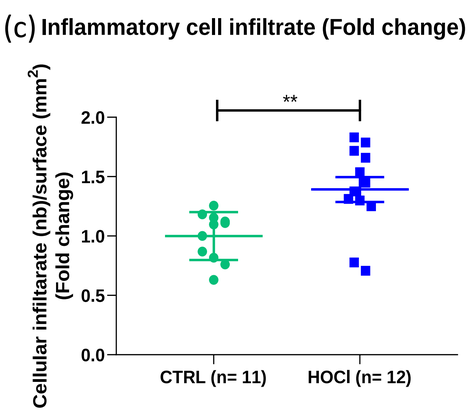


Figure 1. (A) Outline of experimental design. **(B)** FibroNest Method Workflow .

RESULTS

Inflammatory Cell Infiltrates Quantification





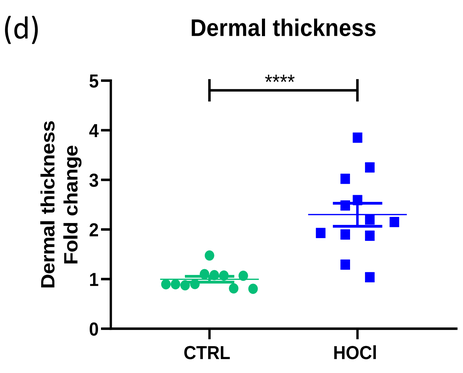
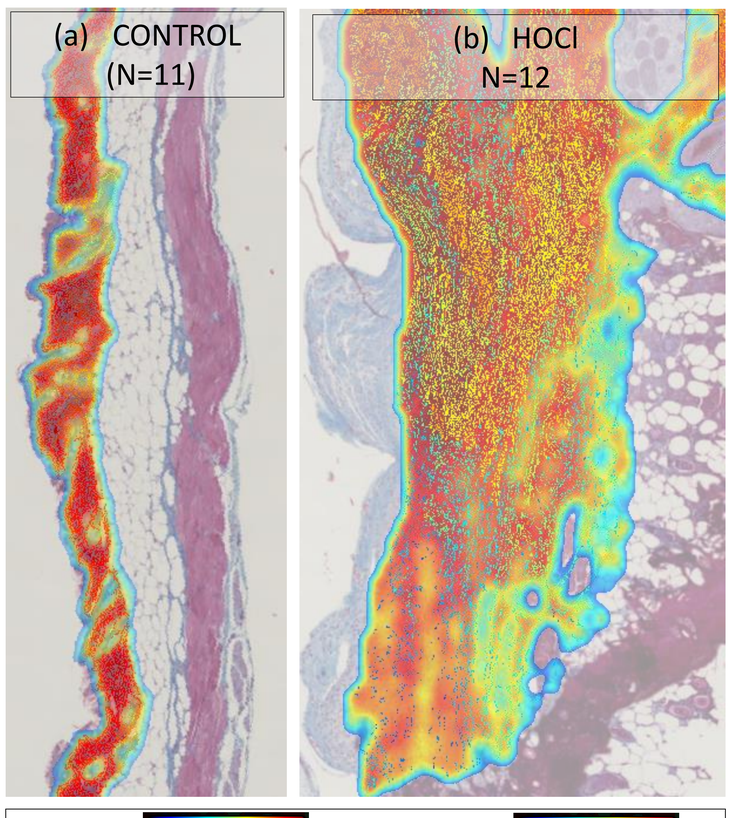
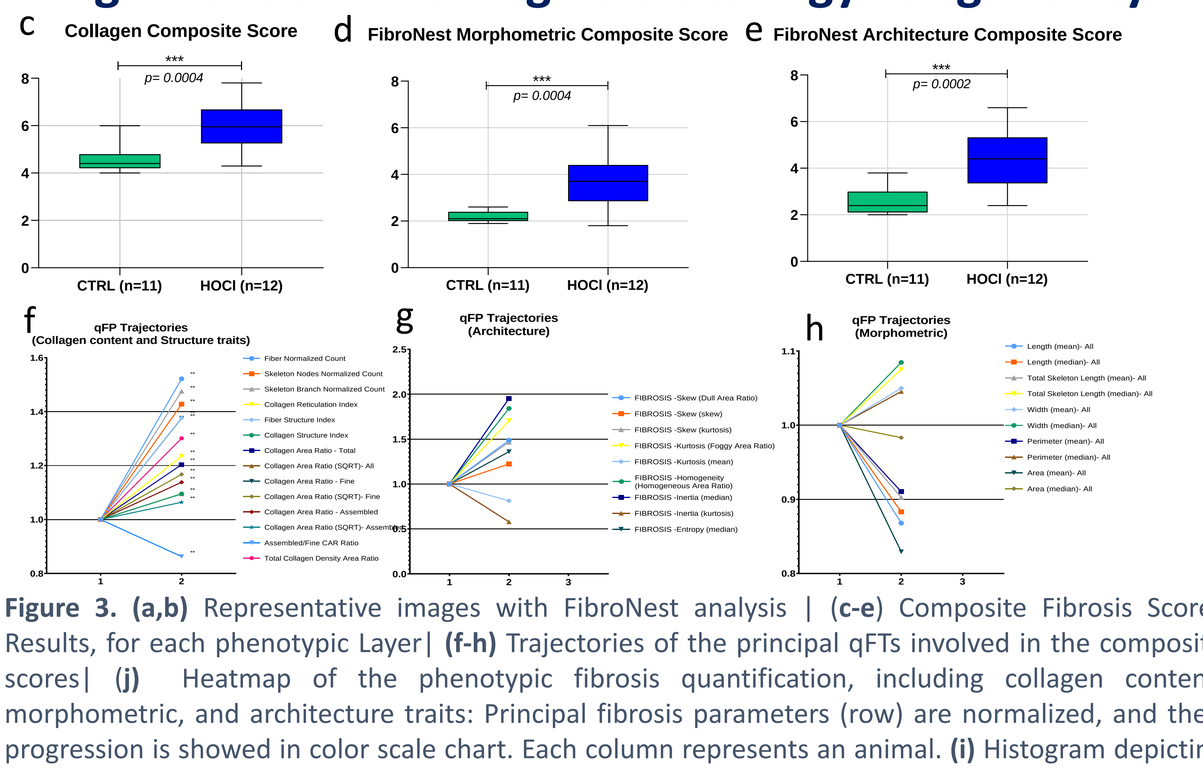
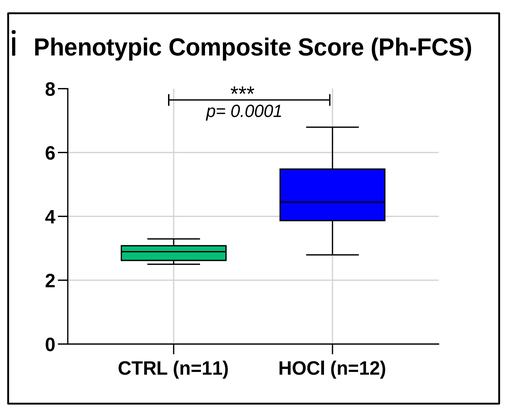


Fig 2. Histological skin evaluation and inflammatory response. (a-b) Representative microphotographs of H&E stained skin sections scanned at 20X magnification. (c) Dermal thickness (d) Normalized count of cellular infiltrates in MGG stained skin sections. (c) and (d) are measured using Image J software. Data represent the fold change compared to CTRL group, are quoted as mean ± SEM and analyzed by non parametric Mann Whitney test; *= p< 0.05; **= p< 0.01

Single-fiber Fibrosis Digital Pathology Image Analysis







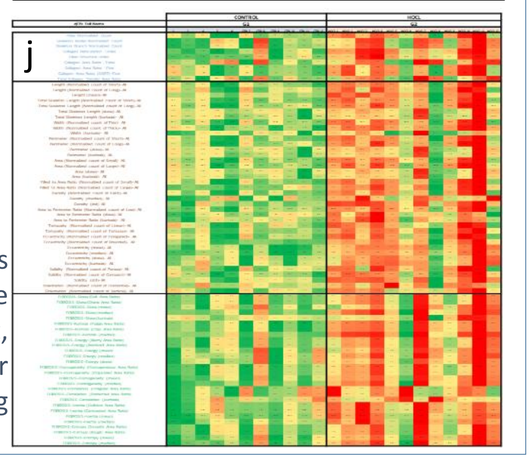


Figure 3. (a,b) Representative images with FibroNest analysis | (**c-e**) Composite Fibrosis Scores Results, for each phenotypic Layer| **(f-h)** Trajectories of the principal qFTs involved in the composite scores| (**j**) Heatmap of the phenotypic fibrosis quantification, including collagen content, morphometric, and architecture traits: Principal fibrosis parameters (row) are normalized, and their progression is showed in color scale chart. Each column represents an animal. **(i)** Histogram depicting Phenotypic Fibrosis Composite Scores (Ph-FCS) deriving from the combination of all parameter

CONCLUSION

The HOCL rodent model recapitulates the hallmarks of Inflammation and Fibrosis of Systemic sclerosis. The use of single fiber Digital Pathology enables the quantification of the histological phenotype of fibrosis in these complex tissues to account for changes in both content and architecture of fibrosis and provide high resolution insights for the investigation of mechanisms of actions. In addition to cellular infiltrates densities, Fibrosis composite scores such as the Skin Ph-FCS might be a relevant readout in future studies on pathophysiology and drug candidate assessment in this disease.

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