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DIGITAL EXPERIENCE

Evaluation of anti-fibrotic effects of compounds in human 3D NASH model using phenotypic quantification of fibrosis digital pathology

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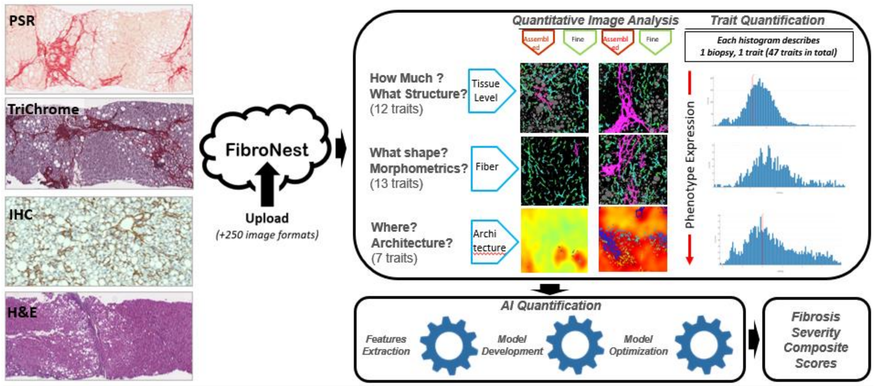
BACKGROUND and AIMS

In vitro human 3D NASH models have the potential to accelerate the discovery of new anti-fibrotic compounds. Until now, *in vivo* evaluation of NASH severity has been based primarily on approximative histological investigation of the deposited fibril collagens within the liver tissues. The fibrotic histological evaluation of stained 3D NASH tissues is complex, and still an emerging field. Automatic and quantitative computerized methods for the evaluation of the fibrotic severity are of high interest. Here, we report that such novel methods can quantify fibrosis severity and treatment response, as well as have the potential to become direct fibrosis endpoints.

METHODS

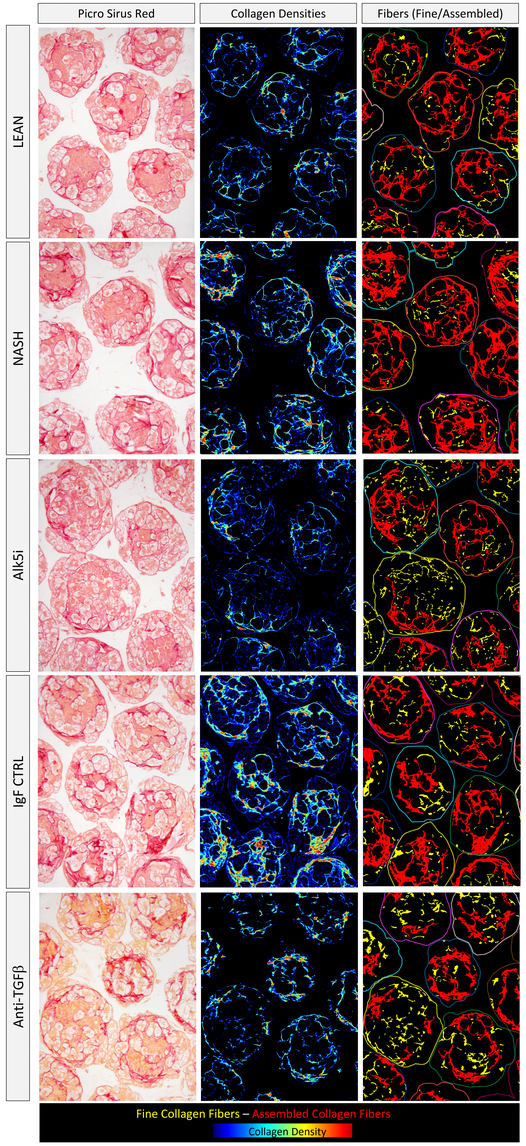


Group	Description	Total =43
LEAN	Human *in vitro* 3D InSight™ liver microtissues containing primary hepatocytes, Kupffer cells, endothelial cells and hepatic stellate cells in 96-well plates	N= 9
NASH	The 3D liver microtissues grown and exposed a defined cocktail of free fatty acids, LPS and high levels of sugars (“NASH stimuli”)	N= 8
Alk5i	Concurrent to the NASH stimuli, the 3D liver microtissues were simultaneously treated with ALK5 inhibitor (TGF-β R1 inhibitor),	N= 7
IgG CTRL	Concurrent to the NASH stimuli, the 3D liver microtissues were simultaneously treated with an isotype control antibody	N=9
Anti-TGF-β	Concurrent to the NASH stimuli, the 3D liver microtissues were simultaneously treated with an anti-TGF-β1,2,3 antibody (0.001 uM).	N=10

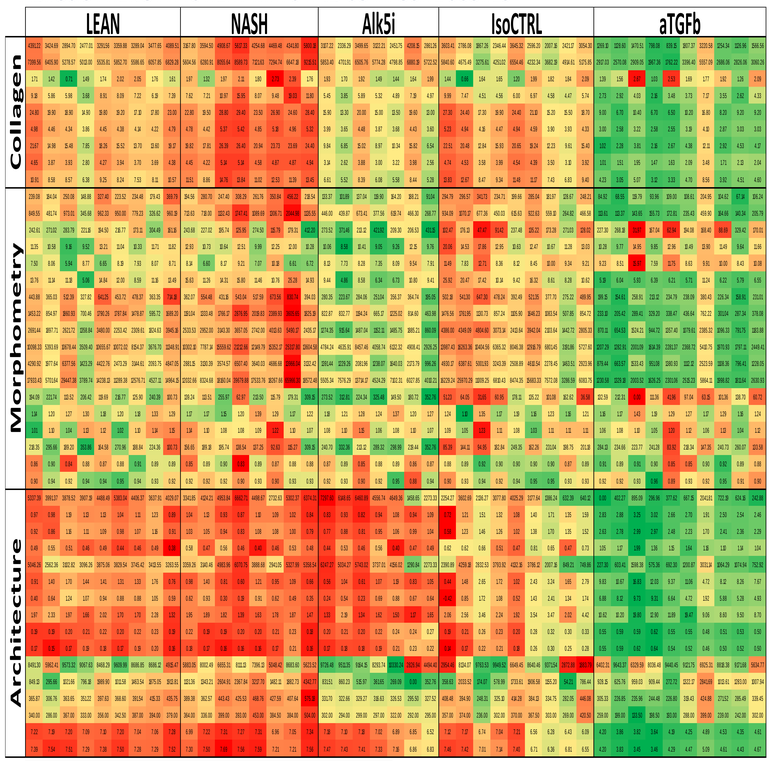
- FFPE sections (~4um) of the pooled liver microtissue colonies were deparaffinized, stained with Masson Trichrome for Collagen and digitized at 40X into an 8-bit TIF flat file image.
- Truncated microtissues were deemed adequate if their area was greater than 60% of the group mean area.
- Using Quantitative Image Analysis (FibroNest™) the fibrosis phenotype was described for its collagen content and structure (12 traits), the morphometric traits of the collagen fibers (13 traits), and fibrosis architecture traits (7). In each image, each morphometric and texture trait were represented by a histogram distribution
- The histogram for each trait was described by up to seven quantitative fibrosis traits (qFTs, 315 in total) to account for mean, variance, distortion and progression. Sub-classes for Fine and Assembled collagens are created based on their reticulation level (defined as the ratio of the fibers skeleton branches and nodes).
- To detect phenotypic signatures of severity, principal qFTs were automatically selected if their group mean value difference (between the LEAN and NASH group) is statistically (P<0.05, T-Test) greater than 20%.
- Principal qFTS were used individually and collectively to describe the phenotypes of fibrosis severity. They were combined into a normalized Phenotypic Composite Fibrosis Score (Ph-CFS), a continuous quantifier of the fibrosis phenotype.

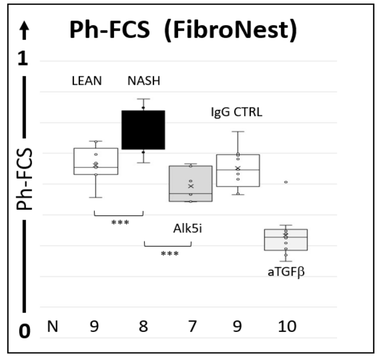
RESULTS

REPRESENTATIVE IMAGES AND FIBRONEST ANALYSES



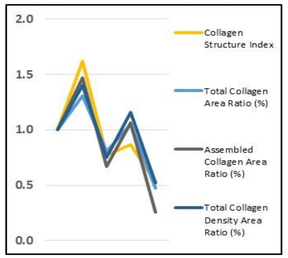
FIBROSIS PHENOTYPIC HEAT MAPS AND COMPOSITE SCORES



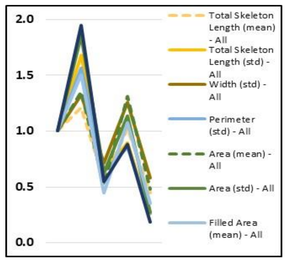


For each microtissue section (column), the Phenotypic maps (above) visualized the relative severity (green to red) of the principal quantitative fibrosis traits (qFTs) as quantified from the image, and were automatically selected to account for variability between groups. Their values were combined (similar weights) to form a Phenotypic Composite score (Ph-FCS) that quantifies fibrosis severity. This score exhibits a superior signal-to-noise ratio that enables a very high detection threshold and remarkable robustness.

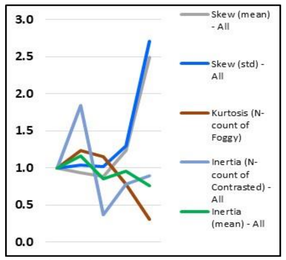
(A) Selected Collagen qFTs



(B) Selected Morphometric qFTs



(C) Selected Architecture qFTs



The Principal qFT trajectories represent the change of the qFT (group mean value) normalized to their NASH value. They provide mechanistic insights.

Conclusion

FibroNest analysis was able to quantify the fibrosis phenotype in the 3D NASH model to account for fibrosis severity and quantify anti-fibrotic treatment response. These data demonstrate the suitability of the method for a histology-based fibrosis endpoint in the 3D NASH model.

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