NASH triple culture validation

005606-001

Supplier: LifeNet health
Test date: January 2023
Experiment: CLT23001

Overview

This report describes the validation of primary human hepatocytes (PHHs), Kupffer cells (KCs) and hepatic stellate cells (HSCs) within the PhysioMimix® NASH-in-a-box kit.

Our quality control procedures ensure that the donor cell combinations within NASH-in-a-box form stable three-dimensional liver microtissues that demonstrate a Non-alcoholic steatohepatitis (NASH) phenotype when cultured over 14 days by PhysioMimix OOC Systems.

Protocol

Tri-cultures of PHHs (lot# 2120321), KCs (lot# 2117722) and HSCs (lot# 2116572) were seeded on day 0 at $4x10^5$ for PHHs and $4x10^4$cells for KCs and HSCs per well into a PhysioMimix Multi-chip Liver-12 plate. The following day the cells were treated with bespoke HEP-Fat media to induce a NASH phenotype. On day 4, cells were treated with 0.5 ng/ml TGFβ or left untreated.

To assess cell health throughout the experiment, LDH and albumin were measured at each media change (Fig 1). The following inflammatory markers; IL-6, IL-8, IP-10, TNF-α, MCP-1, GRO-A and fibrotic markers; procollagen, TIMP-1, YKL-40, fibronectin, were measured on days 8, 11 and 14 (Figs 2, 3 respectively).

On day 14, the scaffolds within each well of the Multi-chip Liver-12 plate were removed, and the microtissues within the scaffold's pores were fixed, labeled and imaged using a confocal microscope (10x magnification) to assess the quality of tissue formation (brightfield imaging. Fig 4), fibrosis and steatosis (fluorescence imaging. Figs 5, 6). The qualification followed our 001154_Rev01 HSC Validation Protocol.

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Validation Data

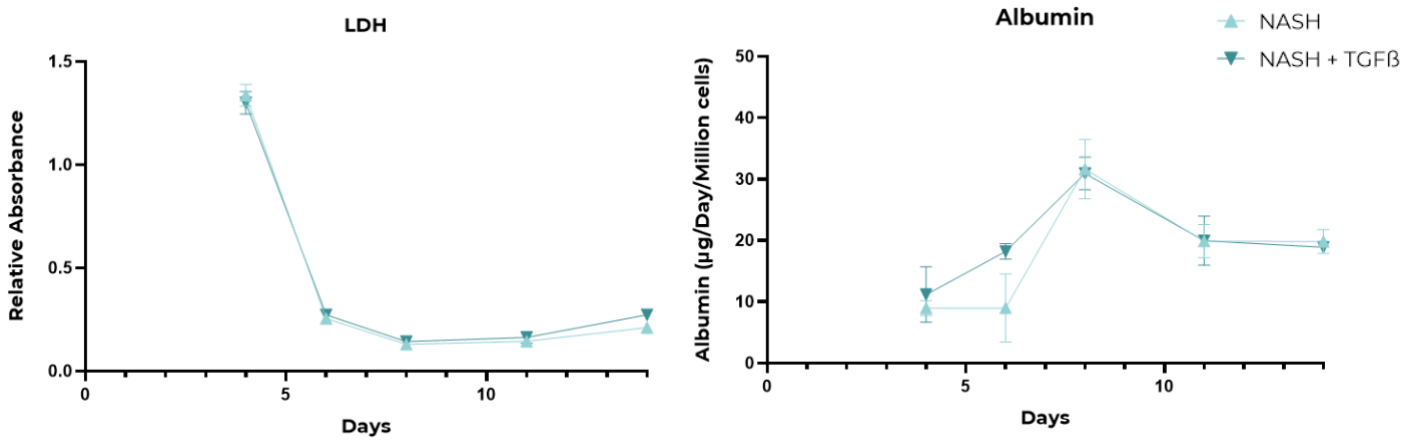

Figure 1. Cell health analysis. *Cell health analysis. Quantification of LDH and albumin levels within media samples at days 4, 6, 8, 11, and 14 of culture by colorimetric assay or ELISA, respectively. Data presented as mean with SD from triplicate samples*

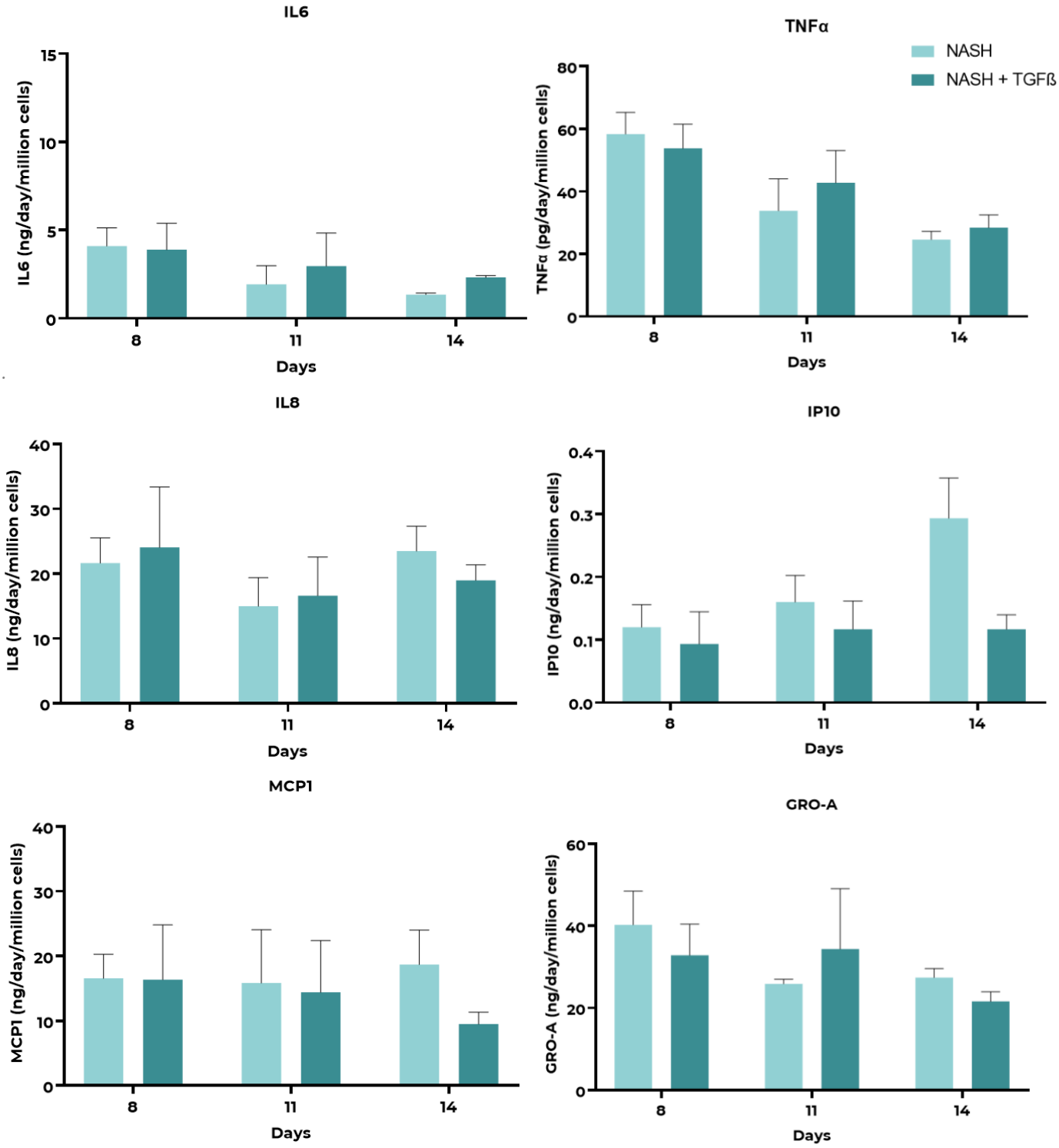

Figure 2. Inflammatory biomarker analysis. *Quantification of IL6, IL8, TNFa, IP10, MCP-I and GRO-A within media samples taken at days 8, 11, and 14 of culture by Luminex analysis. Data presented as mean with SD from triplicate samples.*

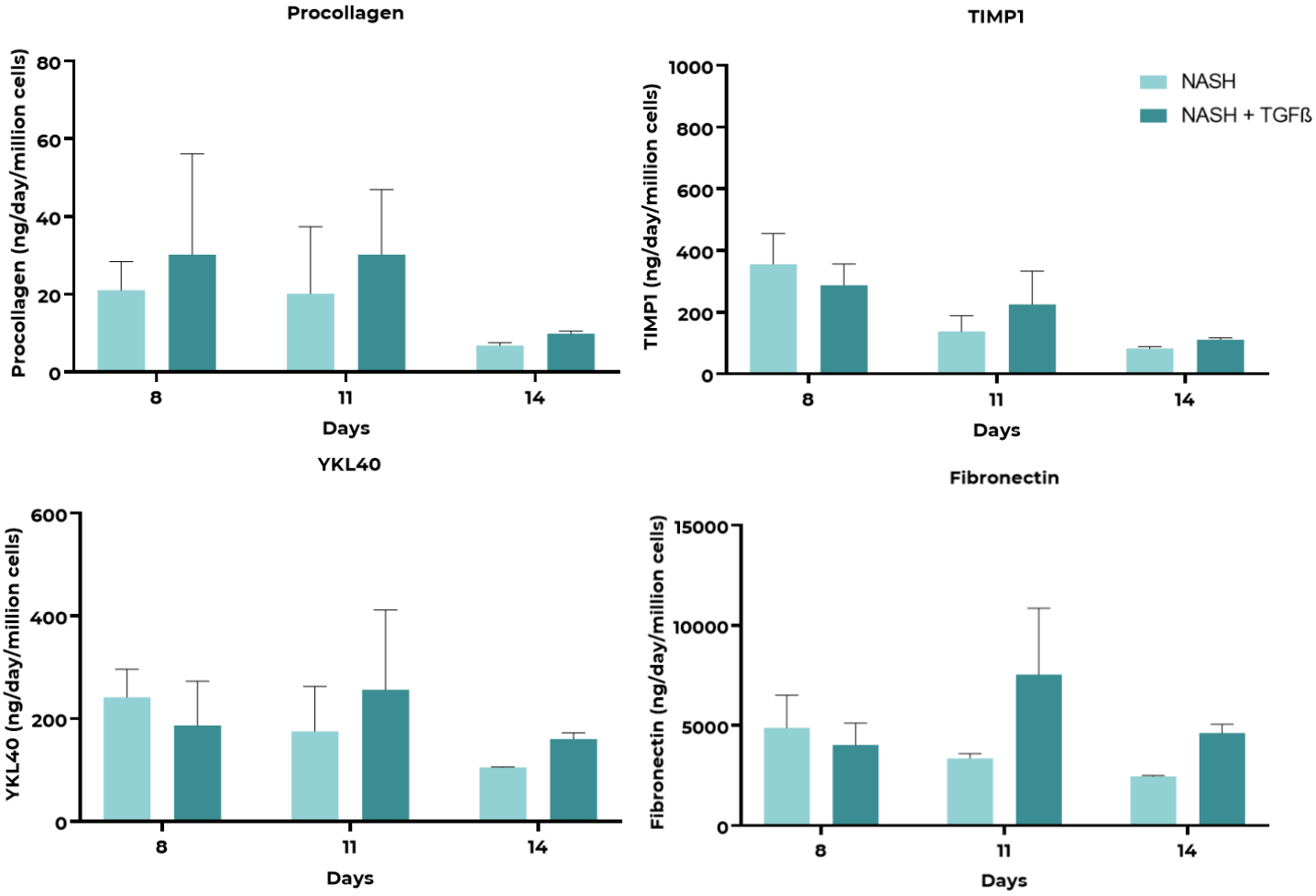

Figure 3. Fibrotic biomarker analysis. *Quantification of fibronectin, procollagen, TIMP-1, and YKL-40 within media samples taken at days 8, 11, and 14 of culture by Luminex analysis. Data presented as mean with SD from triplicate samples.*

Microtissue formation

NASH

NASH + TGFβ

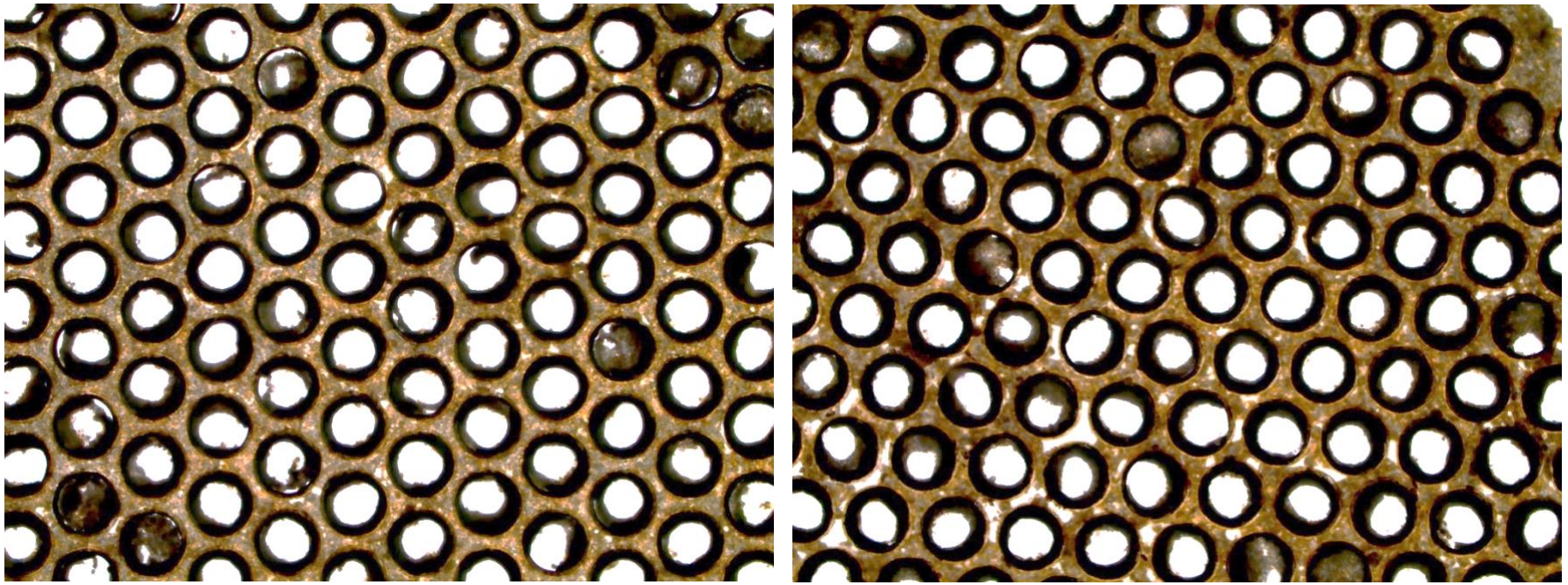

Figure 4. *Brightfield images of the scaffolds in both NASH and TGFß conditions taken at the end of the experiment.*

Confocal Assessment of Fibrosis and Steatosis

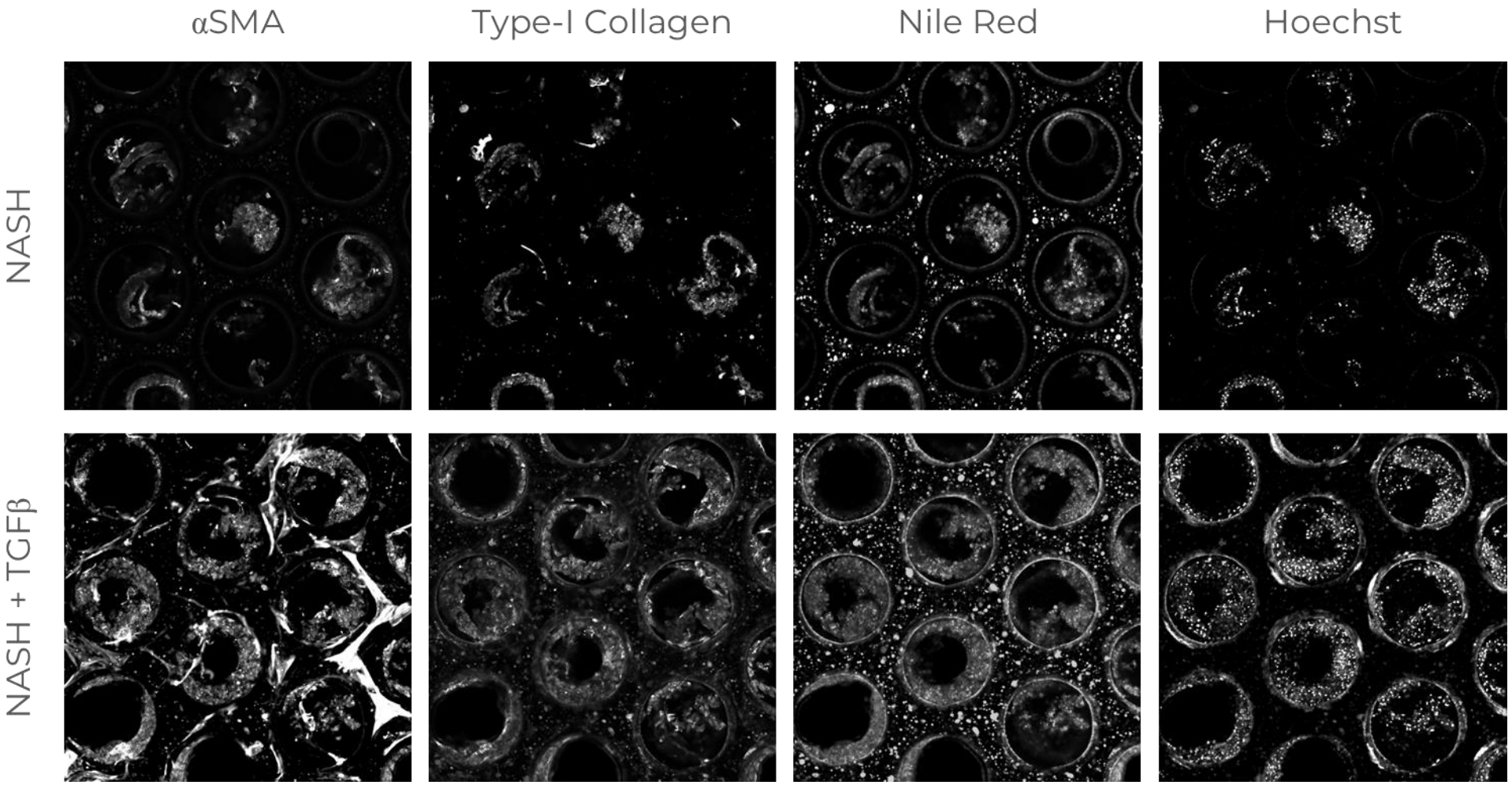

Figure 5. *Example confocal images of the scaffolds in both NASH and NASH + TGFß conditions taken at day 14 at a 10X objective.*

NASH

NASH + TGFβ

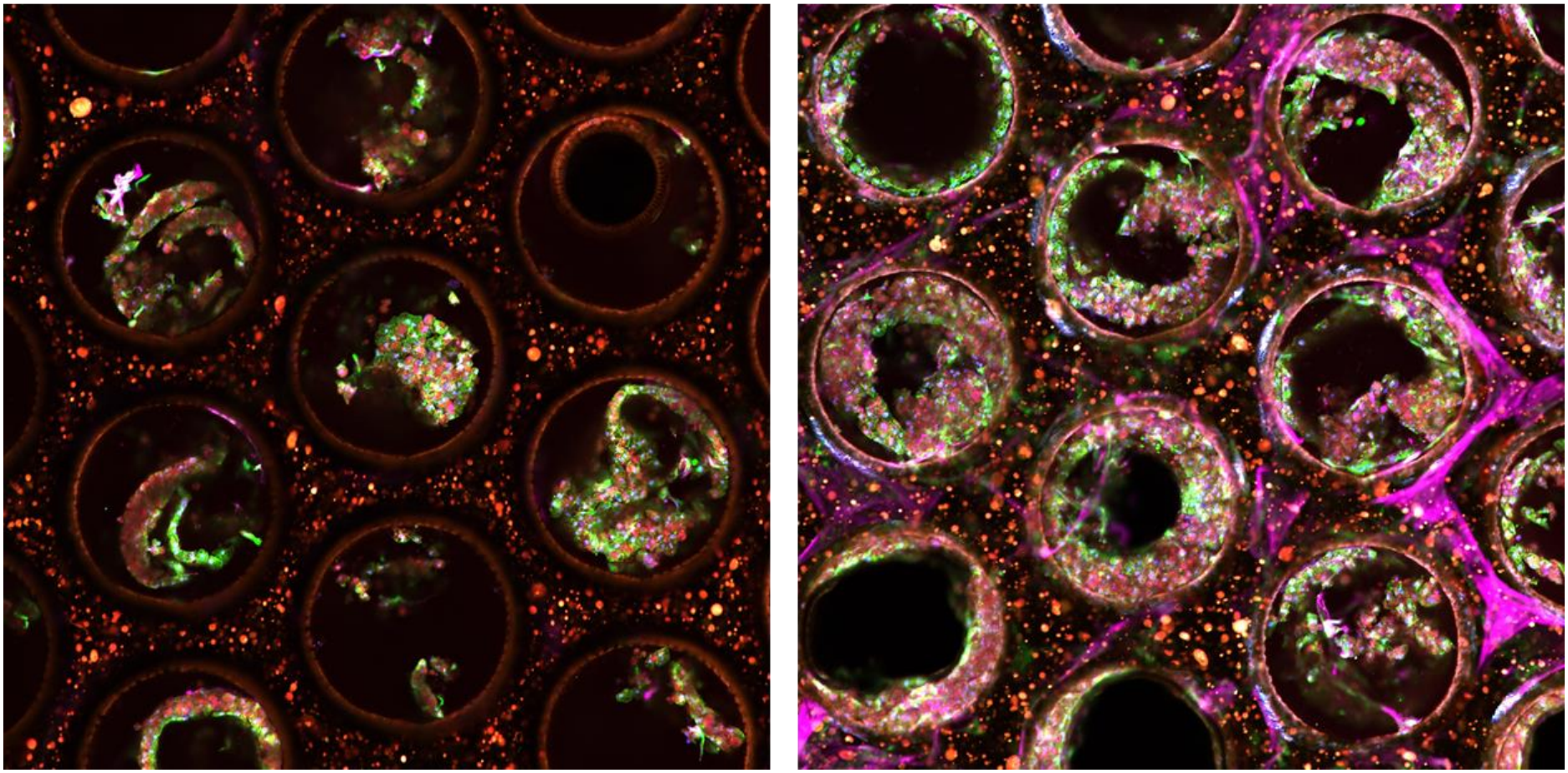

Figure 6. *Confocal composite images of the scaffolds in both NASH and NASH + TGFß conditions taken at day 14. The cells were stained for nuclei using Hoechst (blue), steatosis using Nile Red (red) and fibrosis using αSMA (magenta) and Type-I collagen (green).*