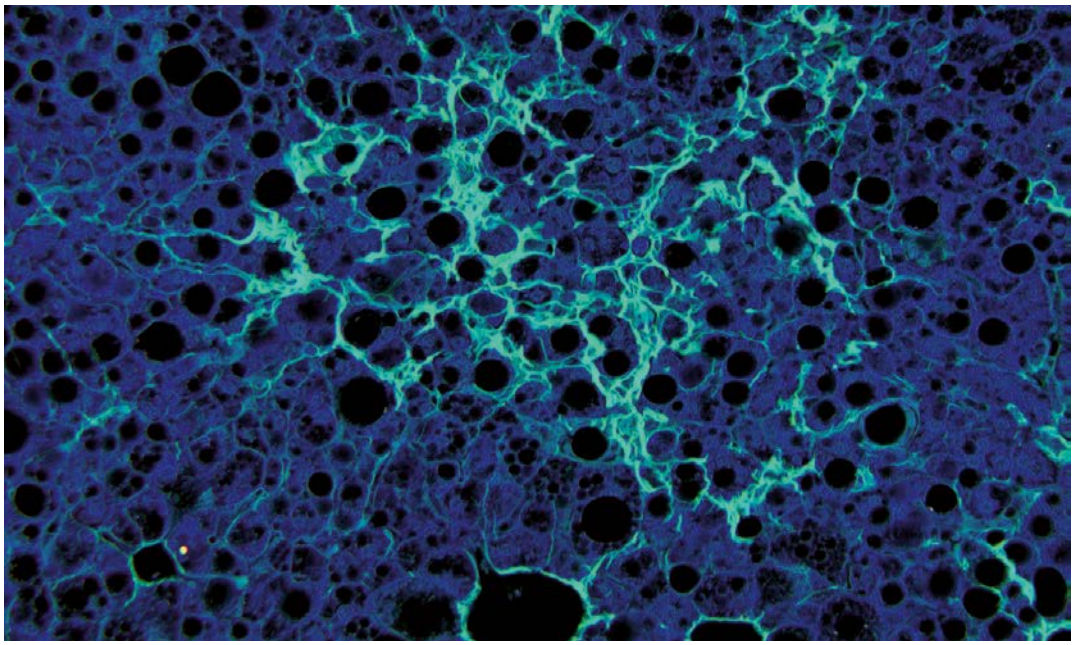
IN VIVO AND *IN VITRO* MODELS FOR FIBROSIS RESEARCH



TNO innovation for life

The recent approval of new therapies for idiopathic lung fibrosis has given an enormous boost to the entire field. Despite these advancements there are still many challenges and opportunities to develop therapies for fibrosis. TNO has extensive knowledge and a large portfolio of *in vivo* (lung, skin, liver and kidney) and *in vitro* models of fibrosis to support the validation of new targets, the testing and optimization of new treatments and to provide insight into the mechanisms of fibrosis.

BLEOMYCIN-INDUCED LUNG FIBROSIS

To study novel compounds against idiopathic pulmonary fibrosis (IPF), we optimized the bleomycin-induced lung fibrosis model in mice. This improved model is induced by oro-pharyngeal administration of bleomycin into the lungs of C57Bl/6 mice. This induces aberrant myofibroblast differentiation accompanied by severe collagen deposition in the alveolar spaces within several weeks. We investigate different aspects of the fibrotic process by studying this model at various time points using histology and collagen content as standard read-outs.
An exciting new read-out is based on the use of deuterated water to label new collagen synthesis. This is especially powerful when testing anti-fibrotic compounds in a therapeutic treatment regimen. In addition, we have time-course gene expression datasets available to study the regulation of your targets in the models.

BLEOMYCIN-INDUCED SKIN FIBROSIS

Skin fibrosis is one of the features of systemic scleroderma. To evaluate novel anti-fibrotic therapeutics, we offer the bleomycin-induced skin fibrosis mouse model. In this model, C57BL/6 mice receive repetitive subcutaneous bleomycin injections into the neck area. This results in dermal thickening and collagen accumulation in the skin over a period of five weeks. The standard read-outs are external skin thickness, dermal thickness and collagen content of the skin. Besides the development of skin fibrosis, this model also features lung fibrosis at later time points, providing the opportunity to study both skin- and lung fibrosis in one model.

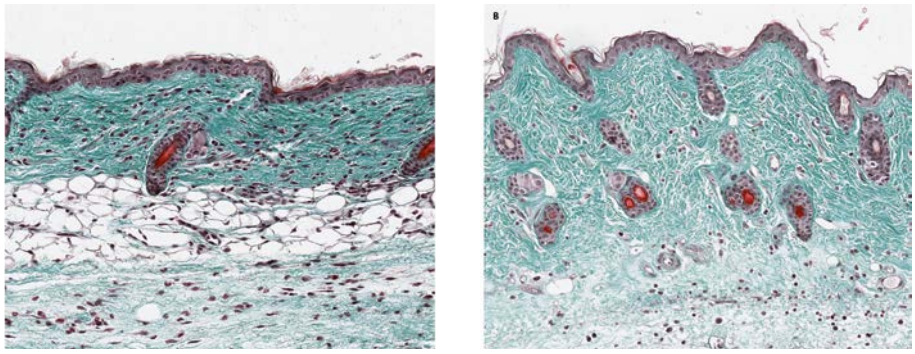


Figure. 1 Histology of bleomycin-induced skin fibrosis in mice. A: normal skin. B: Fibrotic skin showing accumulation of collagen (stained green).

CARBON TETRACHLORIDE-INDUCED LIVER FIBROSIS

We induce liver fibrosis by repeated carbon tetrachloride (CCl_4) injections into C57BL/6 mice. This results in a rapid onset of fibrosis, as characterized by the accumulation of collagen and the presence of alpha-smooth muscle actin positive cells. The model can be run in a preventive setting using a two to four week protocol, but can also be used to test therapeutic treatment regimens.

DIET-INDUCED NASH AND LIVER FIBROSIS

TNO has developed several diet-induced models of NAFLD/NASH and liver fibrosis in which the disease process can be studied in the context of obesity, insulin resistance and dyslipidemia. Animals sequentially develop steatosis, NASH and liver fibrosis including hallmarks of human disease such as infiltration of inflammatory cells, activation of distinct pro-inflammatory transcription factors, formation of cholesterol crystals, and collagen deposition in areas rich in macrovesicular steatosis.

KIDNEY FIBROSIS

We offer the unilateral ureter obstruction model for kidney fibrosis. This model results in a fast (7-10 days) and aggressive induction of kidney fibrosis.
In addition to the standard read-outs, TNO developed a transcriptomics based fibrosis signature to further investigate fibrosis kinetics. Furthermore, we are developing novel models for diabetic nephropathy, which focus on the incorporation of the major metabolic risk factors (obesity, hyperlipidemia, hyperglycemia and hypertension) in a single mouse model.

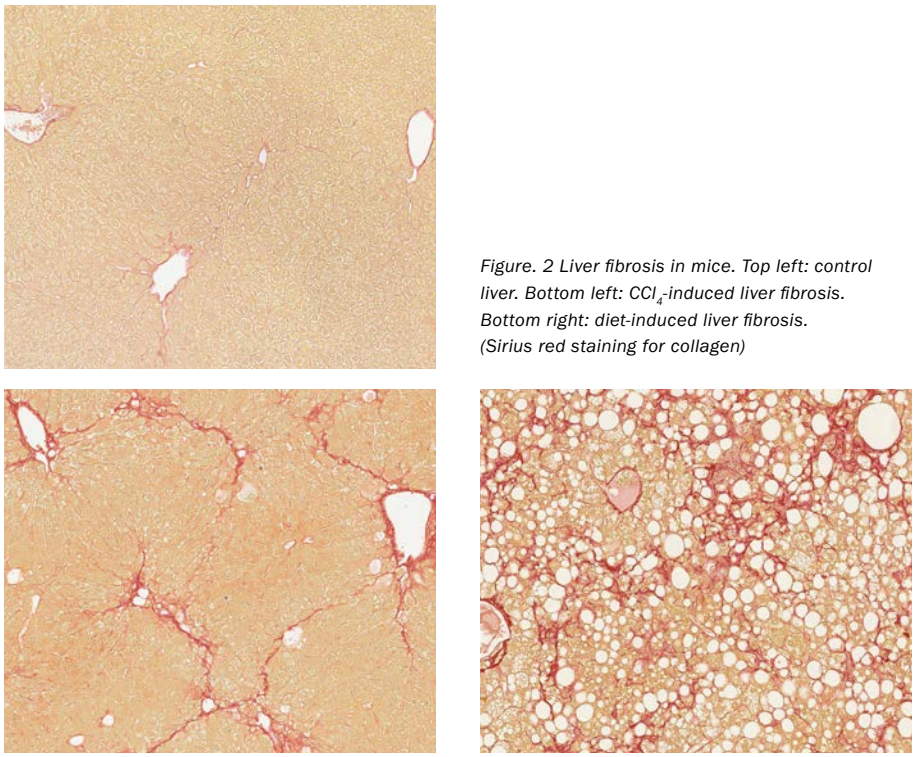


Figure. 2 Liver fibrosis in mice. Top left: control liver. Bottom left: CCl_4-induced liver fibrosis. Bottom right: diet-induced liver fibrosis. (Sirius red staining for collagen)

IN VITRO FIBROSIS

Due to the complexity of the *in vivo* fibrosis process, it is very difficult to mimic this process with a single *in vitro* assay. Therefore, we offer a set of *in vitro* assays, each representing key processes within the fibrotic pathway, such as myofibroblast differentiation, migration, and collagen production. These assays, based on human primary lung or skin fibroblasts are well-suited to evaluate effects of compounds on human targets.

Furthermore, we have an active development program on organ-function-on-a-chip technology. A major part of this program focusses on the development of an *in vitro* steato-hepatitis models using primary human hepatocytes and stellate cells.

ADVANTAGES OF WORKING WITH TNO

- Highly experienced study directors help you optimize the study design according to your needs.
- AAALAC and ISO 9001 certified labs
- We continuously work on further validation and expansion of our portfolios in close contact with industry.

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TNO HEALTHY LIVING
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