

TNO: The power of innovation

Characterisation of Engineered Tissue Constructs

Over the past 10 years considerable progress has been made in the area of in vitro engineering of connective tissue replacements. Many scientists and companies over the world have programmes for engineering cartilage, bone, skin, tendon or ligament replacements.

Tools to characterising the composition of the extracellular matrix are of great value in determining the quality of the engineered tissue constructs. Using our tools, we help you to determine the quality of your engineered constructs of e.g. cartilage or skin.

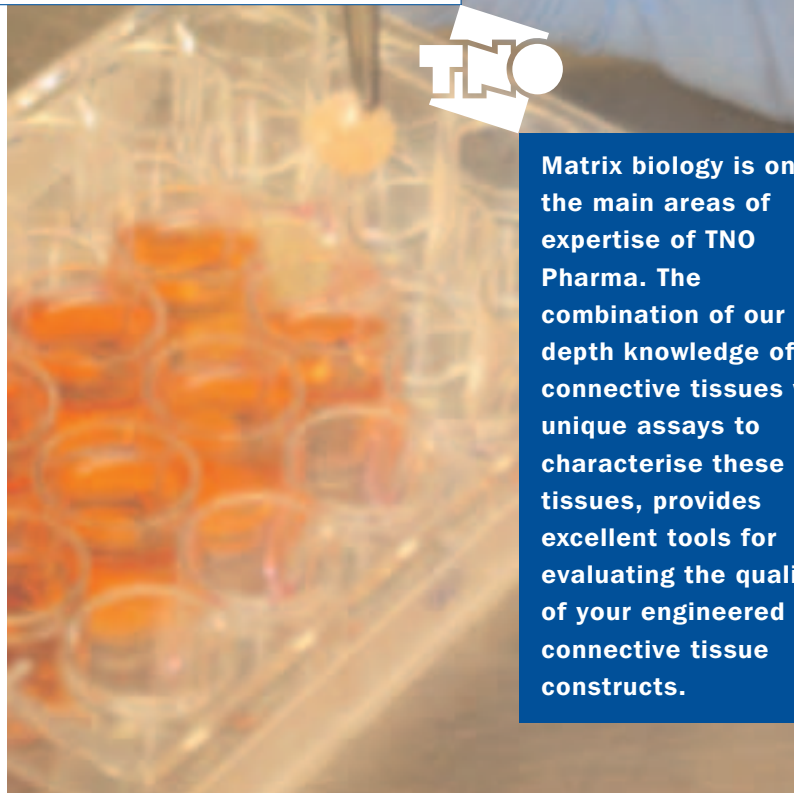
General

Our tools cover characterisation of all components of engineered connective tissue constructs: cells, collagen network, proteoglycans, and (mainly relevant for bone) mineral. Besides measures for these individual components, we also provide general measures of tissue architecture and integrity (like histology). For most species, including the human, reference tissue is available.

A brief description of our capabilities in the characterisation of (engineered) connective tissue is covered in this product sheet. The combination of our in-depth knowledge of connective tissue biology with these laboratory tools opens numerous opportunities to determine the quality of engineered tissue constructs. Therefore, we invite you to contact us for customised information, dedicated to your specific needs.

Cells

- Cell number is determined by measuring the DNA content of the tissue



Matrix biology is one of the main areas of expertise of TNO Pharma. The combination of our in-depth knowledge of connective tissues with unique assays to characterise these tissues, provides excellent tools for evaluating the quality of your engineered connective tissue constructs.

constructs, either using Hoechst 33258 or commercially available kits (e.g. PicoGreen®).

- Gene expression is quantified using real time PCR (collagen, collagen-modifying enzymes, proteoglycans, MMPs).
- In viable tissue, indicators of cell function (e.g. in response to cytokines) are determined (mitochondrial function, MMP production).
- FACS analysis of cell-surface markers and/or intracellular cytokines.

Collagen

- The collagen content is measured as the amount of hydroxyproline (Hyp), either colorimetrically or by HPLC.
- The type(s) of collagen present in a tissue construct can be determined using collagen type specific antibodies (Western blot). The ratio of type I/III collagen is determined using interrupted gel electrophoresis.
- The percentage of denatured collagen is

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 [8] Verzijl N. *et al.*, **J. Biol. Chem.** 2000, 275: 39027-39031.
 [9] Wassen M.H. *et al.*, **J. Bone Miner. Res.** 2000, 15: 1776-1785.

measured by an enzymatic method developed in house using the ability of α -chymotrypsin to digest denatured but not intact triple helical collagen. Further information on the integrity of the collagen network is obtained by determining the swelling of the tissue in hypotonic solution.

- Hydroxylation of Lys residues (Hyl) and glycosylation of Hyl residues (Gal-Hyl, Gluc-Gal-Hyl) are measured by HPLC.
- The enzymatic collagen cross-links hydroxylslypyridinoline (HP) and lysylpyridinoline (LP) are measured by HPLC (see figure 1). A method to quantify difunctional collagen cross-links is currently under development.
- Advanced glycation endproducts (AGEs) result from nonenzymatic modification of proteins by reducing sugars. Several methods are operational to determine well-characterised AGEs (pentosidine, HPLC) and more general measures of AGE modification (absorption at 340 nm, fluorescence at ex 370 nm / em 440 nm, amino acid modification).
- A functional measure of the stability of the collagen network is obtained by measuring its degradability, either by exogenous enzyme addition (MMPs, bacterial collagenase) or by stimulation of the cells to produce collagen-degrading enzymes (using cytokines).
- Measuring the percentage D-Asp assesses the age of the collagen network.

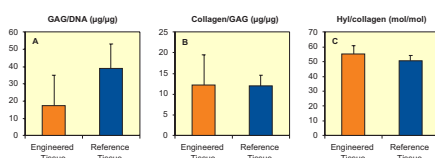


Figure 1. Composition of engineered bovine cartilage constructs (Engineered Tissue) and normal bovine articular cartilage (Reference Tissue). A. Proteoglycan content relative to the number of chondrocytes (GAG/DNA). B. Collagen content relative to the amount of proteoglycans (Collagen/GAG). C. Hydroxylation of collagen Lys residues (Hyl/collagen).

Proteoglycans

- The proteoglycan content is measured as the amount of glycosaminoglycans (GAGs) using a colorimetric assay.
- A functional measure of the stability of the proteoglycans is obtained by measuring their degradability, either by exogenous enzyme addition (MMPs) or

by stimulation of the cells to produce proteoglycan-degrading enzymes like aggrecanases (using cytokines).

- Measuring the percentage of D-Asp (after extraction) assesses the age of the proteoglycans.

Proteases

- MMP activity using fluorogenic substrates; after extraction.
- MMP, cathepsin, and aggrecanase activity using our proprietary Quickzyme™ technology.

Mineral

- Mineral content is measured by colorimetric assessment of the total amount of calcium.
- The percentage of non-mineralized vs. mineralized collagen is measured by heating powdered bone to denature the non-mineralized collagen using the ability of α -chymotrypsin to digest denatured but not intact triple helical collagen.

Tissues

- Connective tissues such as cartilage, bone, skin, tendon, and ligament.
- Our technology is also applicable to other tissues like e.g. kidney, lung, heart, and macula.

Complementary tools

- In vivo (osteo)arthritis and fibrosis models.
- In vitro culture systems of chondrocytes, osteoblasts, and skin fibroblasts.

Track record

Over a decade of experience in research of connective tissue diseases resulted in many studies for pharmaceutical, biotechnology and nutraceutical companies all over the world, including top-10 companies.

Selected references (full list available on request)

[1] Bank R.A. *et al.*, **Matrix Biol.** 1997, 16: 233-243.
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About TNO Pharma

TNO Pharma is a recognized partner for pharmaceutical- and biotech industries and offers a broad array of tailor-made and value-creating services in the discovery and development of drugs and bioactives. TNO Pharma is part of TNO, the Netherlands Organisation for Applied Scientific Research. www.pharma.tno.nl

