Skin regeneration in severe burn wounds: developing signaling scaffolds to prevent fibrosis

Fibrosis is a pathological condition that may affect a plethora of organs; from skin to kidneys, liver and heart. In general terms fibrosis is hallmarked by excessive deposition of matrix, leading to destruction of tissue architecture and thereby compromising normal tissue function. The mechanisms through with fibrosis develops is largely identical for all tissues. ›The process is usually triggered by other diseases or injury, leading to the activation of myofibroblasts which produce extracellular matrix (ECM) and increase tissue stiffness by contracting the surrounding ECM (figure 1). Skin fibrosis may develop after the occurrence of a full-thickness skin injury, when both the epidermis and dermis are damaged. Without clinical intervention, the healing process will result in a fibrotic scar. Especially in the case of severe burn wounds the area of injury can be extensive and may be life threatening if left untreated. Burn wounds are commonly treated with a procedure called 'skin grafting' (figure 2). Aside from donor site morbidities this procedure often leads to severe fibrotic scarring, resulting in painful and mobility limiting scar tissue (figure 3).

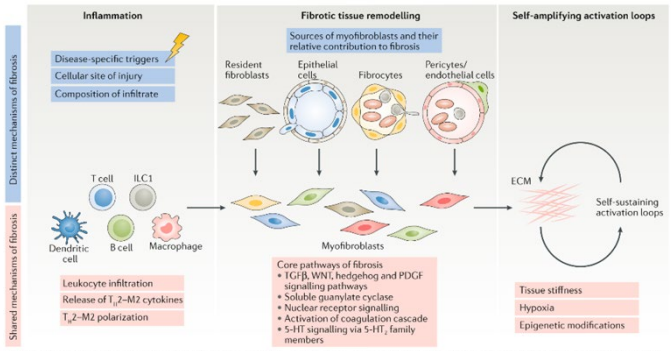

Figure 1. The common mechanisms of fibrosis. Distler, J. H. W. (2019). Nat Rev Rheumatol **15**(12): 705-730.

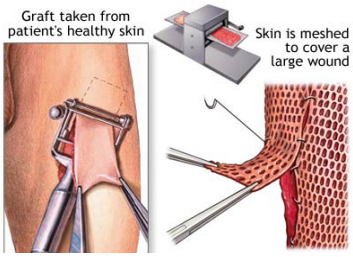

Figure 2. Schematic representation of a skin grafting procedure.

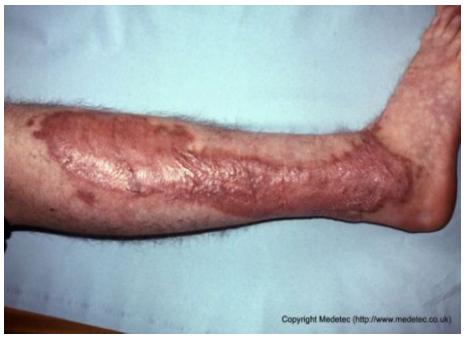

Figure 3. Fibrotic healing after skin grafting.

The key players in the fibrotic response are the myofibroblasts, which become trapped in a self-amplifying activation loop. Their activation is mainly controlled by the transforming growth factor beta (TGFβ) pathway. The TGFβ ligand is excreted by various cells during the first phases of wound healing and the newly activated myofibroblasts start contracting the surrounding ECM. However TGFβ is also stored in an inactive form in the the ECM, known as the latency complex. Contraction of the ECM leads to release of TGFβ from the latency complex, further stimulating the myofibroblasts. For years researchers have been developing methods to achieve tissue regeneration by suppressing the fibrotic response. Generally research has focused on the application of scaffold material to promote cell ingrowth or on the delivery of cells and growth factors.

This research project will combine multiple methods to limit myofibroblasts and achieve regeneration, focusing on spatial control and sustained activity of growth factors. To achieve this, collagen scaffolds will be functionalized with novel heparan sulfate mimetics, known as regenerating agents (RGTA). Collagen scaffolds provide structural support for the healing tissue and allow for ingrowth of cells. The collagen also functions as a carrier for the RGTA, which bind and protect growth factors but have the benefit of degradation resistance. To the functionalized scaffold two well-known anti-fibrotic growth factors will be added: fibroblast growth factor 2 (FGF2) and hepatocyte growth factor (HGF).

Many techniques are envisioned in the project, including biomaterial production and assessment, cell culture, SDS-PAGE, Western blotting, ELISA, immunostainings and RT-qPCR. The student will produce and optimize the collagen-RGTA scaffolds, as well as determine the ability of the scaffolds to immobilize growth factors. The amount of RGTA bound to the scaffolds will be investigated using the Farndale biochemical assay and histology. Growth factor immobilization will be confirmed through SDS-PAGE, Western blotting and ELISA. The *in vitro* biological activity of the signalling scaffolds will be evaluated using cell culture models of myofibroblasts. The fibrotic response will be visualized through immunostainings and RT-qPCR.

The internship will be hosted at the department of Biochemistry, in the group of Dr.ir. Willeke Daamen and Dr. Toin van Kuppevelt, daily supervision will provided by a PhD candidate. We are looking for a motivated MSc student with a special interest in regenerative medicine. The intended starting date is in the autumn of 2021 and the project will run for a minimum of 5 months. If you are interested in this position, please submit a CV and

letter of motivation to Merel Gansevoort (merel.gansevoort@radboudumc.nl), potential candidates will be invited to an interview.