Project title: Imaging of collagen structure and its linkage sites to other proteins of the extracellular matrix by multimodal nonlinear microscopy

Supervisors: Simone Baltrusch (UROS), Albert Stolow, Ravi Bhardwaj, Paul Corkum (uOttawa)

Current state of the art

Collagen is the most important and quantitatively most abundant structural protein in the human body [1]. It is a component of the so-called extracellular matrix, which in addition to collagen also consists of elastic fibres, proteoglycans, glycoproteins, and adhesive molecules. Collagens themselves represent a heterogeneous structural group, which is divided into different types and classes [1]. The largest of these are the fibril-forming collagens, in addition to which there are the fibril-associated collagens, in which the periodicity of the triple helix is interrupted, basement membrane collagens, network-forming collagens, microfibrillar collagens and transmembrane collagens [1]. Many of these collagen structures take on specific properties in the human body and are not interchangeable. Since collagens have a long half-life, remodelling processes, in which part of the aged structure is always replaced by new ones, are highly coordinated processes. If the collagen structure is damaged by external influences such as accidents or inflammatory processes, the remodelling process is disrupted and properties such as tensile and compressive strength cannot be maintained. Since type 1 collagen, which belongs to the fibrillar collagens, is the most common collagen, ex vivo production is now well advanced and its use in the manufacture of vascular grafts and tissue replacement material has been promoted [2]. The aim here is to accelerate wound healing and maintain stability and organ function until the body's own remodelling process reaches it again [3]. Currently, such structures are being tested for their mechanical properties such as tensile strength and compressive stability. Furthermore, structural analyses are aimed at, which are of central importance in monitoring changes in collagen-based scaffolds [2]. Such changes could already be visualized by anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) microscopy, fluorescence lifetime microscopy (FLIM) and non-linear imaging [2, 3, 4, 5, 6]. In the latter, a combination of two-photon fluorescence and second harmonic generation (SHG) [4], which exploits the non-centrosymmetric structure of collagen, turns out to be very promising. Based on changes in the collagen structure in different disease patterns, it becomes clear that not only a decrease but also a structural change can result in a loss of function. Here, changes in the cross-linking of collagen with other proteins plays an important role. These contact points have not yet been visualized by nonlinear microscopy. However, while SHG requires the specific asymmetry of the collagen structure, third harmonic generation (THG) can also be found at structural interfaces and is thus suitable to identify interactions between e.g. water and collagen bundles or extracellular matrix and collagen scaffolds [7, 8].

Research goals and working program

As mentioned above, the molecular organization of collagen allows for specific imaging using SHG. This was first shown for the fibril-forming collagens and assumed to be specific [4]. Recently, we have succeeded in visualizing the network-forming collagen structure by SHG as well [9]. This proves that possibly already the tropocollagen (triple helix) structure is enough to achieve a signal and the fibril structure based on it only leads to a signal amplification (Fig. 1. By varying the excitation wavelength, the MPE-RS microscope (EVIDENT/Olympus) in combination with the Insight (Spectra Physics) laser made it possible to record the two-photon signal in parallel and to image water-containing proteins of the extracellular matrix [9, 10, 11] (Fig.2).

The aim of this project is to further investigate the structure of collagen by implementing additional modalities. In particular, (1) in vivo and in vitro collagen structures will be compared, (2) transformation processes in collagen will be documented and (3) collagen and other proteins of the extracellular matrix will not only be visualized in parallel, but also cross-linking to other proteins of the extracellular matrix will be investigated. Here, (3a) THG, (3b) FLIM and (3c) CARS and SRS will be applied.

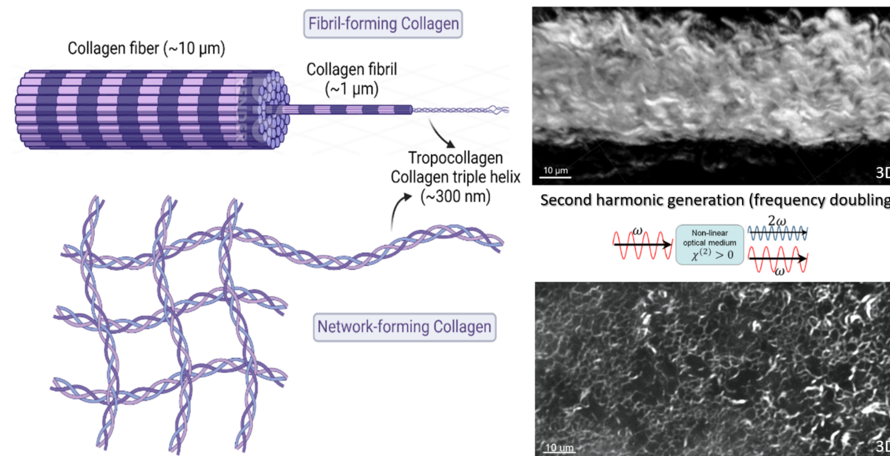

Figure 1: Schematic view of collagen structure (created in BioRender.com) and microscopy images showing fibril-forming collagen (top) and network-forming collagen (bottom) visualized using second-harmonic generation (SHG).

1) Murine organ preparations will be used as the in vivo model. As in vitro model, the following methods of preparation will be applied: Hydrogels, freeze-drying, electrospinning, 3D bioprinting. The analysis will be done by nonlinear imaging using different modalities. [Month 01-12 / Rostock]

(2) Transformation processes in collagen will be studied on pathological changes in murine organ preparations and in vitro. The latter will be achieved by treatment of the synthesized collagen matrices with matrix-metal proteinases. These enzymes also control the remodelling process of collagen in the human body. Analysis is then performed by nonlinear imaging using a choice of different modalities. [Month 13-24 /Rostock]

(3a) THG will be applied as another modality in nonlinear microscopy. For this purpose, organ preparations will be further investigated to identify contact sites to non-collagen proteins of the extracellular matrix. [Month 06-30 /Rostock]

(3b and c) In comparison to (3a) it will be investigated which interactions can be visualized by FLIM and CARS/SRS. For this purpose, new techniques will be developed and tested in exchange with the Canadian partner. [Month 31-36 /Canada]

[1] S. Ricard-Blum, *The collagen family*, Cold Spring Harb. Perspect. Biol. **3**, 004978 (2011).

[2] T. A. Shaik, J. L. Lagarto, E. Baria, M. Goktas, P. I. Onoja, K. G. Blank, F. S. Pavone, J. Popp, C. Krafft, and R. Cicchi, *Monitoring changes in biochemical and biomechanical properties of collagenous tissues using label-free and nondestructive optical imaging techniques*, Anal. Chem. **93**, 3813 (2021).

[3] L.E. Iannucci, C.S. Dranoff, M.A. David, and S.P. Lake, *Optical imaging of dynamic collagen processes in health and disease*, Frontiers in Mechanical Engineering **8**, 855271 (2022).

[4] X. Chen, O. Nadiarynkh, S. Plotnikov, and P. J. Campagnola, *Second harmonic generation microscopy for quantitative analysis of collagen brillar structure*, Nat. Protoc. **7**, 654 (2012).

[5] W. Min, C. W. Freudiger, S. Lu, and X. S. Xie, *Coherent nonlinear optical imaging: Beyond uorescence microscopy*, Annu. Rev. Phys. Chem. **62**, 507 (2011).

[6] C.-Y. Chung, J. Boik, and E. O. Potma, *Biomolecular imaging with coherent nonlinear vibrational microscopy*, Annu. Rev. Phys. Chem. **64**, 77 (2013).

Imaging of collagen structure and its linkage sites to other proteins of the extracellular matrix by multimodal nonlinear microscopy