TIME-LAPSE SHG IMAGING OF COLLAGEN FIBRILLOGENESIS AND CORRELATION TO ELECTRONIC MICROSCOPY

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Type I collagen is a major structural protein in mammals and shows highly structured macromolecular organizations specific to each tissue. This biopolymer is synthesized by cells as triple helices, which self-assemble outside the cells into fibrils that further form fibers, lamellae or other three-dimensional (3D) networks. This assembly mechanism depends critically on the collagen concentration, as well as on the temperature, pH and ionic strength of the solution in vitro. Thorough characterization of collagen fibrillogenesis is crucial to understand the biological mechanisms of tissue formation and tissue remodeling and to design new biomaterials. Advanced in situ quantitative imaging techniques are therefore required to verify whether the tissue substitutes display appropriate biomimetic 3D organization for cell culture scaffolds or functional implants.

In this study, we continuously monitored the formation of collagen fibrils by using time-lapse in situ Second Harmonic Generation (SHG) microscopy¹, and we correlated these data to Transmission Electron Microscopy (TEM) performed at discrete times. SHG microscopy is a nonlinear optical technique that provides specific 3D images of unstained collagen samples with sub-micrometer resolution²,³. Fibrillogenesis was triggered by increasing the pH in a dilute solution of collagen I and the increase of the fibril density was quantified in the SHG image stacks. Our results showed reproducible dynamics of fibrillogenesis that could be changed by tuning the pH. We also monitored the growth of single fibrils and measured the length increase over time, which had never been reported before using an optical technique. We then correlated these SHG images to TEM images by blocking the fibrillogenesis at early stages and drying the samples. It confirmed the formation of striated fibrils with 67nm periodicity like in biological tissues and it showed that SHG microscopy allows imaging of fibrils with a diameter down to 30-50 nm in our experimental conditions. We also investigated surface-mediated fibrillogenesis by adding silica nanoparticles to the solution⁴. We used Two-Photon excited fluorescence (2PEF) microscopy to visualize the fluorescently-dyed nanoparticles and quantify the self-assembly of collagen around these nanoparticles.

In conclusion, SHG microscopy enabled sensitive and well contrasted 3D visualization of collagen fibrillogenesis. It therefore appears as a powerful technique for in situ monitoring of collagen biomaterials in a non invasive way.