TWO PHOTON EXCITATION MICROSCOPY AND SHG IMAGING AS A TOOL FOR VISUALISATION OF TYPE I AND TYPE II COLLAGEN

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Type I collagen is present in fibrous tissue (e.g. tendon) and fibrocartilage, whereas type II collagen proves hyaline character of cartilage. The goal of our experimental work was to explore the capabilities of nonlinear microscopic techniques using second harmonic generation (SHG) imaging and two-photon excitation (2PE) to distinguish type I and type II collagen.
Healthy cartilage and tendon tissues from rabbit and rat as well as repaired articular cartilage were fixed with frozen methanol, immunocytochemically labelled with monoclonal antibodies against type I and type II collagen and subsequently labelled, respectively, with Alexa Fluor 633 and Cy3 conjugated secondary antibodies. In addition, autofluorescence of both native and fixed samples was detected. Co-localization between one-photon excitation (1PE) fluorescence of specifically stained collagen, SHG imaging, and 2PE autofluorescence was studied by Leica TCS SP2 confocal laser scanning microscope, in this study using lasers for 1PE with 488 nm, 514 nm, 643 nm, 633 nm lines and a laser for 2PE at 960 nm wavelength. First, in unlabelled samples we verified that there is no autofluorescence signal detected at 1PE wavelegths of 543 nm and 633 nm, used for excitation of Cy3 and Alexa Fluor 633, respectively. This was shown for both cartilage and tendon. In cartilage, autofluorescence was detected only at 1PE of 488 nm and 514 nm and in tendon only at 488 nm excitation. Using 2PE with wavelegth of 860 nm the cartilage samples showed autofluorescence in the range of 500-620 nm and 680-730 nm while in tendon autofluorescence was detected only in the range of 680-730 nm. In both cases a very strong SHG signal was detected in the range 425-435 nm. In contrast to 1PE and 2PE fluorescence imaging, SHG signal could be detected from deeper parts of the samples. SHG signal and type I collagen colocalized on the tissue surface while SHG signal and type II collagen colocalization was found in the deeper parts of the tissue. However, immature repaired cartilage showed only partial colocalization of SHG signal with type I or type II collagen labelling. SHG signal from type I collagen displayed fibrous structure while SHG signal from type II collagen was more homogeneous.
We conclude that detection of type I and type II collagen using SHG and 2PE autofluorescence imaging is a promising method for determination of the hyaline character of cartilage in tissue engineering applications.

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