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Case Report

Second harmonic generation microscopy to investigate collagen configuration: a pericarditis case study

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Abstract

We have used second-harmonic-generation (SHG) to image collagen fibers in pericardial tissue removed from a patient with constrictive pericarditis and compared this to healthy pericardium. SHG imaging allowed for the visualization of collagen fibers without the need for staining or pretreatment. Images were compared to stained histology slides. Collagen fibers in SHG and histology images displayed the same structure and morphology. The mature collagen of the parietal pericardium was easily distinguishable from the new collagen accumulation due to the pericarditis. SHG imaging can provide a convenient and valuable architectural profile of collagen organization.

Keywords: Pericarditis; Collagen; Diagnostic imaging; Connective tissue

1. Clinical history

The patient initially presented with an umbilical hernia and abdominal distention. An abdominal echo showed ascites and a hepatic condition. The 26-year-old male patient was referred to our hospital diagnosed with constrictive pericarditis and fibrosis-causing restrictive cardiac syndrome. The patient was neither a smoker nor diabetic, with known allergies to penicillin and clarithromycin. The patient did not respond to the prescribed steroids and thus underwent a pericardectomy via stenotomy at our institution. After the surgery, the patient rapidly lost 30 lb and progressed without any complications. A follow-up echocardiogram revealed the patient's heart was functioning and he was discharged home after 8 postsurgical days.

2. Methods and results

Second-harmonic-generation (SHG) microscopy uses nonlinear photon scattering caused by the noncentrosymmetic assembly of collagen to create an image. It can provide fibril resolution without extrinsic dyes [1,2]. SHG also allows for optical sectioning and the ability to image at various depths in excised tissue, due to the deep penetration of the infrared (IR) excitation laser wavelengths and the nonlinear intensity dependence of the SHG emission.

Small pieces of diseased and healthy pericardial tissue measuring 5×3 cm, from the anterior wall of the pericardial sac, were collected for SHG imaging and for histology. The healthy pericardial tissue was removed during coronary artery bypass graft surgery from a 66-year-old male. The samples were first imaged by SHG microscopy (homebuilt) and then sent to histology. SHG images were collected in the *x*-*y* plane and imaged through the tissue depth (*z*-plane) creating *z*-stacks (available online). Both

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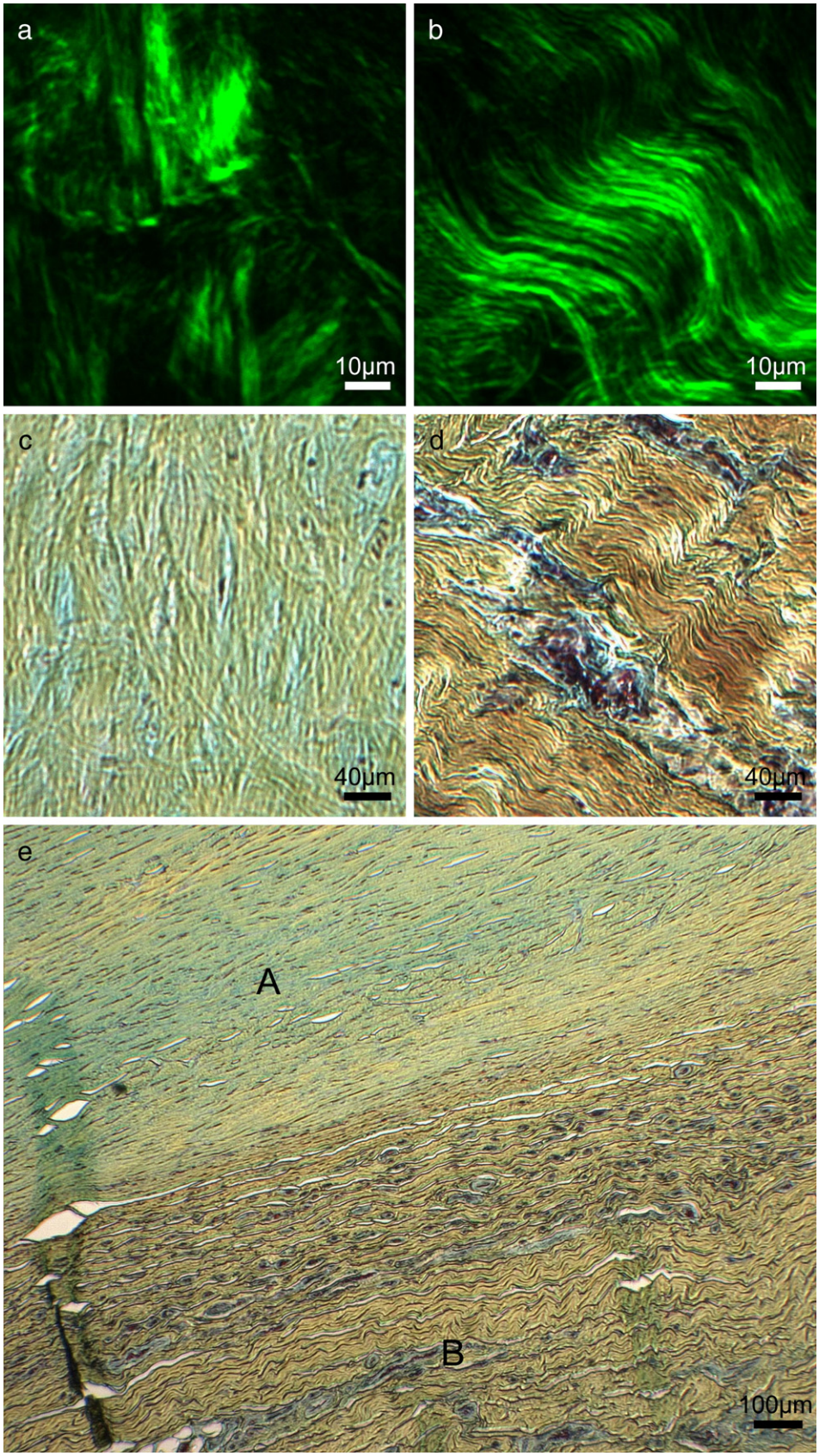
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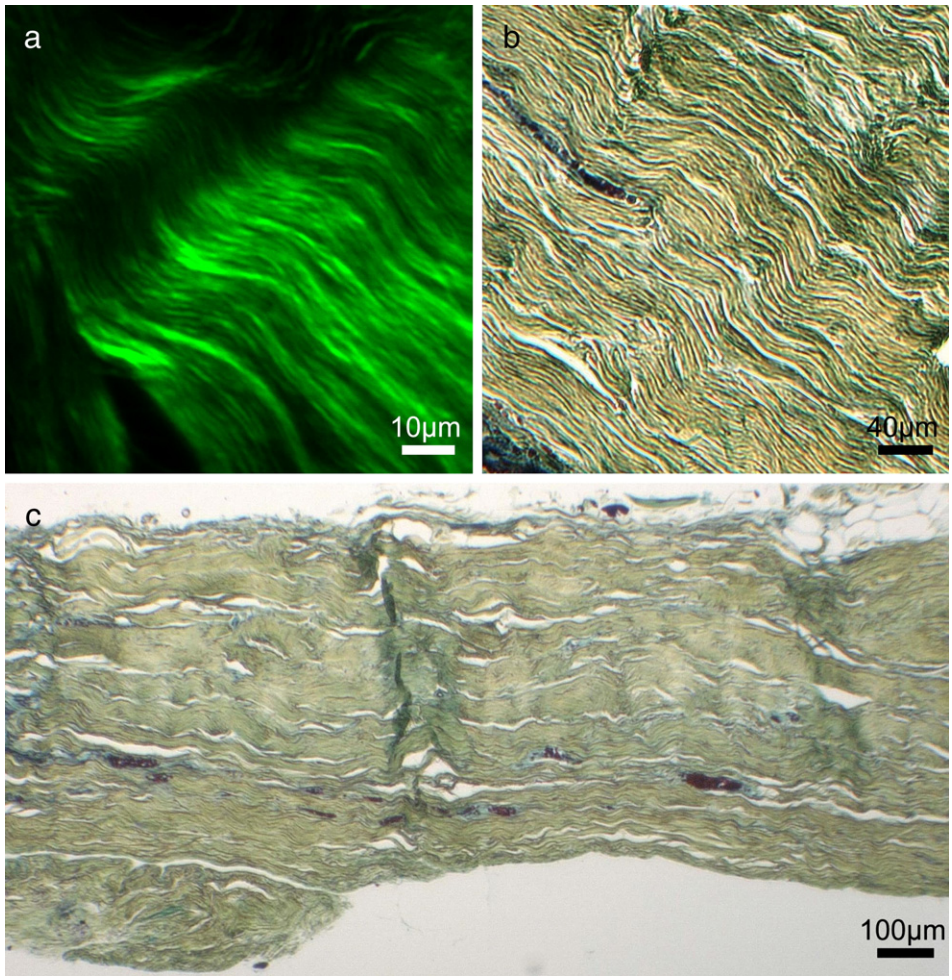

Fig. 2. Healthy pericardium. Comparison of SHG and histology images of a healthy pericardium. (a and b) Pericardium in the superior part imaged en face by SHG (a) and bright field light microscopy (b). (c) The histology cross-section does not show a difference in the collagen orientation throughout the whole depth of the pericardium. The thickness of the healthy pericardium is considerably smaller than the pericarditis case.

cross-sections and en face histology slices (cut throughout the tissue depth) were stained by Movat pentachrome stains. Further SHG images were then taken from the histology slides to ensure that the location within the tissue was known and comparable to the histology images. Histology images were collected using a light microscope (Leica Microsystems, Germany). A digital thickness counter (Litematic VL-50A, Mitutoyo, Japan) was used to measure the tissue thickness at several locations. The values were averaged and are presented as mean value with standard deviation.

The tissue from the pericarditis of the anterior wall was significantly thickened. Upon removal, this part measured 3.47±0.74 mm. Two distinct layers were identified from the cross-section of the pericardium (Fig. 1e), delineated by the maturity of the collagen. Histology and SHG en face images from the parietal pericardium (Fig. 1a and c) showed loosely packed collagen fibers that lacked the typical mature collagen wavy (collagen crimp) morphology. The external layer, which is the original portion of the pericardium, had collagen fibers with the normal wavy morphology (Fig. 1b and d). Few inflammatory cells were visible throughout the tissue however there was increased vascularization. A three-dimensional reconstruction shown in Video 1 (see Online Supplementary Material) shows the change in collagen orientation from the external to the internal layer of the pericardium over 80 μm. Fig. 2 shows a healthy pericardium specimen. This tissue specimen was much thinner 0.44±0.14 mm. The collagen fibers were crimped and consistent throughout the tissue. Some blood

Fig. 1. Pericarditis pericardium. SHG shows a difference in collagen organization of the parietal pericardium of a patient with pericarditis. (a and c) Internal layer of pericardium, imaged en face by SHG (a) and bright field light microscopy (c). The depth at which the images were taken is showed in (e) by A. (b and d) External layer of pericardium, imaged en face by SHG (b) and bright field light microscopy (d). The position at which the images were taken is denoted by B in (e). (e) The histology cross-section shows a clear difference in the collagen orientation from the top and bottom part of the pericardium which can also be seen by SHG (a and b).

components were visible, but there was no indication of inflammation or neovascularization.

3. Discussion

SHG microscopy is a quick imaging tool that is non-destructive and allows for clear visualization of collagen fibers throughout connective tissue without any staining or pretreatment. When compared to histology images there is good agreement between the collagen morphology and orientation. A three-dimensional reconstruction of collagen fibers is possible with this technique which allows for the depth of the tissue to be examined and easily reveals details and spatial understanding which would require multiple histology slices. SHG microscopy is powerful tool for studying tissue structure, and with continued research and improvements in resolution, a variety of applications will benefit. It can be used to measure collagen fibrils down to the submicron scale which is useful for understanding the progression of diseases related to the extracellular matrix [3]. Tissue obtained from biopsies could be quickly evaluated. Eventually, an SHG endoscope could be used to visualize collagen structure in vivo, a prototype for which has already been developed and tested in animals [4].

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