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Poster Communications: Assessment of cardiac fibrosis in hypertrophic hearts using second harmonic generation

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Chronic pressure overload leads not only to hypertrophy but can also result in fibrosis of the myocardium, ultimately leading to diastolic dysfunction. Fibrosis results from increased cardiac fibroblast (CF) proliferation and collagen synthesis, both of which can be quantitatively assessed in normal and diseased myocardium. The excess collagen produced by CFs in fibrosis is a birefringent material. Using a suitable laser source, it is possible to generate a harmonic wavelength from collagen and to collect this signal as a contrast mechanism in order to study collagen density within a three-dimensional specimen. This approach may be superior and more selective than previously used methods, such as histological staining, as no disruptive chemical labels are required. Here, we optimise the use of second harmonic generation (SHG) microscopy for imaging collagen in left ventricular (LV) tissue sections from normal and hypertrophic hearts. A minimally invasive transverse aortic banding (MTAB) mouse model was used to induce LV hypertrophy. Mice (C57, 25-30g) were anaesthetised with 3% Isoflurane in oxygen, maintained with 1.5% Isoflurane in oxygen, and were given analgesic (60µg/kg Buprenorphine) intramuscularly. Resulting hypertrophy and contractile

dysfunction were assessed by increased heart weight:body weight ratios (9.4±0.6 cf. 5.1±0.3, n=9, p<0.0001, MTAB vs sham) and decreased % fractional shortening (22.6±2.2 cf. 42.7±2.1, n=9, p<0.0001, MTAB vs sham). Importantly, a hyperproliferative phenotype was evident in CFs isolated from MTAB hearts in response to 1μM Angiotensin II stimulation (59.6±2.5 cf. 41.8±2.3 (mean cell count per field measured), n=3, p=0.006, MTAB vs sham). Collagen deposition was assessed in LV sections using a multi-photon laser scanning microscope, capturing both two-photon excited autofluorescence (TPEF) and SHG images simultaneously in two channels. The femtosecond-pulsed laser was tuned to 900nm for excitation of LV sections, and the two channels recorded emission in the wavelength range of 480-700nm and 450±2.5nm for TPEF and SHG signals, respectively. Collagen deposition was significantly increased in MTAB hearts (121.5±28.6 cf. 32.9±4.7 (relative intensity, AU), n=5, p=0.0157, MTAB vs sham). Verification of the suitability of this novel approach was confirmed using parallel traditional picrosirius red staining, which was markedly increased (6.31±1.2 cf. 1.45±0.2 (% area of staining in field measured), n=7, p=0.0014, MTAB vs sham,). In comparison with traditional histological staining, SHG/TPEF appears a sensitive tool for collagen characterisation in heart tissue. This novel and non-destructive imaging method will allow more accurate assessment of the extent of fibrosis occurring during disease progression. Crucially it will provide an invaluable screening tool for testing novel anti-fibrotic treatments.