Jonas Golde, Luise Schreiber, Patricia Gerstner, Anett Jannasch, Ralf Schallert, Jörg Opitz, Frank Sonntag, Malgorzata Kopycinska-Müller, Sems-Malte Tugtekin, Claudia Dittfeld

Detection of fiber structure and calcification in xenogeneic pericardium by OCT

An innovative option for biomaterial testing in cardiac surgery

https://doi.org/10.1515/cdbme-2025-0143

Abstract: Aortic valve (AV) stenosis is the most treated heart valve disease and the only curative treatment is the surgical AV replacement or the transcatheter prosthesis implantation. Bovine and porcine pericardium is used for fabricating biological heart valve prostheses but the durability is limited to 10-15 years. We use optical coherence tomography (OCT), a volumetric imaging technique with microscopic resolution, to monitor fiber structure and calcification of pericardium samples. Validated by histology, OCT at 880 and 1300 nm central wavelength captures the relevant structural features and shows a high potential for material testing of pericardium towards improved AV prostheses.

Keywords: xenogeneic pericardium, optical coherence tomography, polarization, calcification, material analysis, biological aortic valve prosthesis, histology

1 Introduction

Aortic valve (AV) stenosis is the most treated heart valve disease and still the replacement of the diseased aortic valve in cardiac surgery or the implantation of a biological prothesis via transcatheter technology (Transcatheter aortic valve implantation, TAVI) are the only treatment strategies [1]. In approx. 90 % of the surgical interventions and for all TAVI procedures biological aortic valve prostheses are used [2]. Biological AV prostheses are fabricated from bovine and porcine pericardium but also from porcine aortic valves [3]. Therefore, the tissues are fixed in glutaraldehyde (GA) solutions in most of the preparation strategies [4]. Implantation of biological AV prostheses does not necessitate long-term anticoagulation therapy for the patients [1]. Nevertheless, bioprothesis degeneration after 10-15 years results in functional loss and the need for a second substitution performed on the one hand by a second surgical procedure or on the other hand by valve-in-valve TAVI [5]. Bioprothesis degeneration with matrix calcification and functional loss is beside aspects of biomechanics caused by free aldehyde groups remaining after GA-fixation or by immunological reactions triggered by xenogeneic antigens such as α-Gal [4, 6]. As a result, research focus is the development of alternative preparation strategies leading to a prolonged durability of the bioprotheses [4, 7]. Prior to *in vivo* biocompatibility testing and clinical studies, these preparation protocols and fabricated tissues have to be validated in detail *in vitro* [4, 8] described also in DIN EN ISO 5840 und 10993. One possibility for material testing strategies is the analysis of *in vitro* calcification using calcification inducing solutions such as simulating body fluid [9-11]. Combining these concepts with dynamic incubation in micro-physiological systems coupled to optical coherence tomography (OCT) is envisioned to monitor initial calcification process. OCT, a non-invasive volumetric imaging technique with microscopic resolution, offers already the possibility of biological sample analysis in the biomedical field such as examining the retina, the characterization of coronary plaques or the discrimination between fibrous, lipid and calcified plaques [12-15]. In addition to the standard OCT contrast based on back-scattered intensities, polarization changes in a sample are further explored in polarization-sensitive OCT (PS-OCT) setups [12, 16]. Both OCT analysis of xenogeneic pericardium and resulting *in vitro* calcified tissue is presented with the aim to initially investigate applicability for material testing concepts.

***Corresponding author: Jonas Golde:** Fraunhofer Institute for Material and Beam Technology IWS, Dresden, Germany; **jonas.golde@iws.fraunhofer.de**
Luise Schreiber: Fraunhofer Institute for Ceramic Technologies and Systems IKTS, Dresden, Germany
Patricia Gerstner, Anett Jannasch: Department of Cardiac Surgery, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Heart Centre Dresden
Ralf Schallert, Jörg Opitz: Fraunhofer IKTS, Dresden
Frank Sonntag: Fraunhofer IWS, Dresden
Malgorzata Kopycinska-Müller: Fraunhofer IKTS, Dresden
Sems-Malte Tugtekin, Claudia Dittfeld: Department of Cardiac Surgery, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Heart Centre Dresden

2 Materials and Methods

2.1 Pericardium preparation and histological evaluation

Bovine and porcine pericardia were purchased from local slaughterhouses (Vorwerk Podemus Dresden, Germany; Fleischland Sora, Klipphausen, Germany). GA-fixation was performed for 4 h at room temperature (RT) using 0.625 % GA diluted in 20.4 mM HEPES-buffer supplemented with 13 mM $MgCl_2 \times 6\ H_2O$ and 80.6 mM NaCl (pH 7.4). GA-fixed porcine pericardium was incubated in calcification solution "G&W" [9]. In brief, equal volumes of solution A (7.754 mM $CaCl_2$*$2H_2O$ in Tris-buffer, pH 7.4) and solution B (4.642 mM K_2HPO_4 in Tris-buffer, pH 7.4) were mixed prior to incubation or fluid renewal. Pericardium (approx. 1 cm^2) was incubated in 2 ml of the solution at 37°C in a rotator for 30 days with medium renewal every second day. Pericardium samples were fixed, paraffin embedded and sectioned (3 µm) for histology using hematoxylin/eosin (HE), picrosiriusred, alcianblue, Elastica van Giesson von Kossa and alizarinred stain. Porcine pericardium was calcified using a published protocol [9].

2.2 Optical coherence tomography

OCT imaging was performed using two commercial spectrometer-based devices at central wavelengths of 880 nm (Thorlabs Ganymede GAN332 with OCTP-900 and OCT-LK2-BB) and 1300 nm (Thorlabs Telesto TEL211PS with OCTP-1300PS and OCT-LK4). The configurations were chosen to provide a high resolution at 880 nm (2.2 µm axial, 4 µm lateral) and a larger field of view with additional polarization contrast at 1300 nm (5.2 µm axial, 20 µm lateral resolution). Tissues were placed on glass slides in PBS buffer and additionally covered by a second cover glass. Slightly tilting the scan probe reduced oversaturation from surface reflexes and improved sample contrast. Volumes of different sizes were acquired while sufficient lateral sampling with respect to the spot sizes of the setups was always ensured. All Ganymede volumes were processed using the manufacturer's software (ThorImageOCT 5.8.0, Thorlabs) and Fiji/ImageJ for visualization. Raw PS-OCT data was processed using custom scripts and visualization tools (MATLAB R2024b, MathWorks). For additional polarization contrast, the cumulative retardation and the degree of polarization (DOP) were calculated [16] and merged with the intensity data by a color-encoded visualization. If two scale bars are presented, the vertical indicates axial size at a refractive index of 1.35.

3 Results

3.1 OCT of xenogeneic pericardium

The fiber structure of bovine pericardium can be visualized via 1300 nm but also in the 880 nm system OCT system for the entire cross section (Figure 1). Using the 1300 nm, the fiber orientation is mainly visualized by the polarization contrast based on the cumulative retardation and especially highlighted in the enface section with a high directionality (right). ECM fibers are depicted in the histological evaluation using HE and especially picrosiriusred stain, which visualizes the wave-like collagen fibers (P). Acianblue (AB) stain reveals a low content of glycosaminoglycans. The elastin fiber orientation apparent

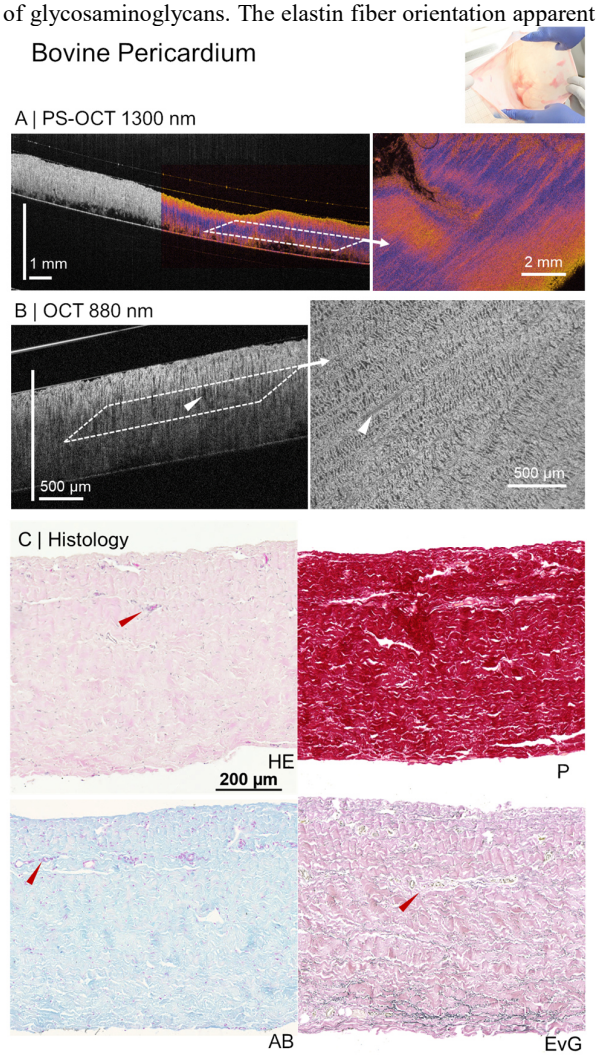

Figure 1: OCT analysis (A,B) of GA-fixed bovine pericardium and histological verification (C) of general ECM fiber structure and sporadic vessel distribution (HE, hematoxylin & eosin; P, picrosiriusred; AB, alcianblue; EvG, Elastica van Giesson).

after Elastica van Giesson (EvG) staining is heterogeneous over the tissue cross section with sprawled fibers (upper part of the section) vs. transverse fiber cut (lower part of the sections). Pericardium is interstratified with small vessels (examples marked with red arrows in HE, AB and EvG, Figure 1). These structures can be related to low intensity areas in OCT analysis of the bovine pericardium (white arrow in B). Porcine pericardium usually exhibits a third of the tissue thickness of bovine pericardium and the entire tissue could be visualized with both OCT systems (Figure 2). The results are comparable to bovine pericardium, but the dense tissue limits imaging the fiber structure at a high contrast in both tissue orientations. Nevertheless, PS-OCT slightly improves the visualization of the organized tissue, based on the cumulative retardation. Also, porcine pericardium exhibits wave-like collagen fiber structure in histological analysis in HE and picrosiriusred stain. Different layers of fiber orientation seem due to the tissue thickness not that pronounced as in bovine tissues. A low level of glycosaminoglycans were detected in alcianblue stain of porcine pericardium and elastin fiber orientation seem to be more homogeneous than in bovine pericardium (EvG stain is more intense due to an adoption in protocol).

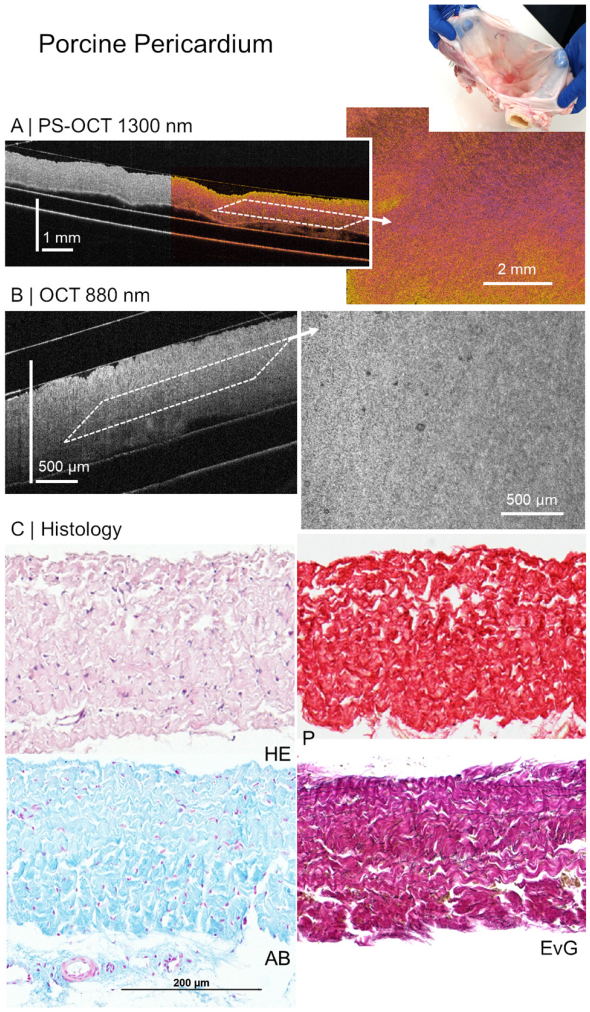

Figure 2: OCT analysis (A,B) of GA-fixed porcine pericardium and histological verification (C) of general ECM fiber structure and sporadic vessel distribution (HE, hematoxylin & eosin; P, picrosiriusred; AB, alcianblue; EvG, Elastica van Giesson).

3.2 Calcification of porcine pericardium in OCT analysis

OCT analysis of the calcified porcine pericardium revealed a surface covering of the tissue with mineral phase that exhibit different and therefore inhomogeneous OCT intensities compared to tissue matrix (Figure 3). A nearly complete covering of one side of the pericardium was visualized with higher OCT intensities and partially calcification was monitored on the opposite tissue border. This can be related to remaining connective tissue since preparation of pericardium does not guarantee complete removal in every location. As an example in the alcianblue stain histology remaining connective tissue is shown. Nevertheless, also in the ECM calcification can be visualized, which is especially highlighted in the color-encoded DOP (Figure 3A, white arrows). As shown in Figure 3B, a mineralic layer (small white arrows) did not impair the possibility to investigate the entire tissue cross section. By imaging from both sides (big arrows indicate direction), the same structures (+,*) can be identified.

Histologically, calcification is usually monitored using alizarinred (AR) and von Kossa (vK) stain [21]. Also, the calcification of porcine pericardium after *in vitro* calcification in the respective solution can be monitored using this analysis revealing the covering of the tissue surface but also matrix calcification at least in part of the ECM.

4 Discussion

Xenogeneic pericardium mainly consists of type I collagen and elastic fibers (collagen 72-76% and elastin, 4-5%; [17]). Compared to porcine aortic valve leaflets the content of glycosaminoglycans is very low [18]. Native pericardium is anisotropic and exhibits different mechanical properties in different directions, GA-fixation results in a higher grade of isotropy [19, 20]. Based on both conventional OCT and the additional polarization contrast, the anisotropy of the tissue could be resolved. Strikingly, OCT at 880 nm with high resolution resolves the characteristic fiber structure, while this not directly assessable at lower resolution with 1300 nm.

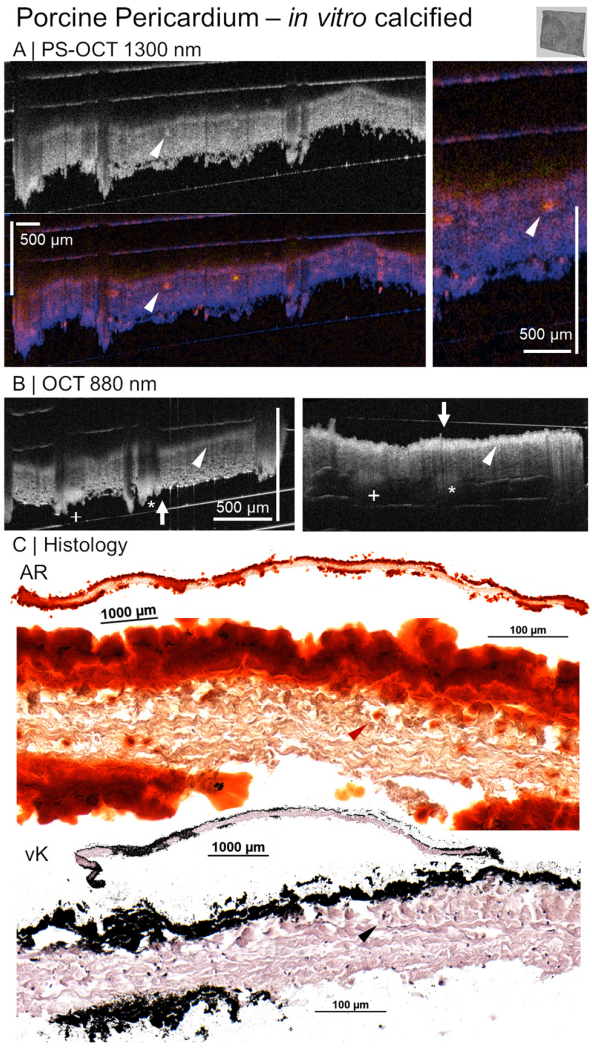

Figure 3: Visualization of *in vitro* calcification of GA-fixed porcine pericardium via OCT analysis (A,B) of GA-fixed porcine pericardium and histological verification (C) of calcification (AR, alizarinred; vK, van Kossa).

Porcine pericardium was *in vitro* calcified using a fluid published by Golomb and Wagner in 1991 [9]. Although this can be discussed critically and other calcification inducing solutions have been introduced to better reflect the process *in vitro* [10, 11], the reliable hydroxyapatite tissue internalization was the aim. Nevertheless, mineral precipitation on the tissue surface might be at least part of the process and was expected. Indeed, OCT showed such a surface covering. Here, especially the DOP is a promising polarization contrast for *in vitro* monitoring and investigation the calcification, also in dynamic incubation platforms, which is facilitated by the real-time cross-sectional imaging capabilities of OCT.

5 Outlook

Investigating xenogeneic pericardium via OCT illustrates the applicability of the technique for the assessment of tissue fiber structure, which is validated by the comparison to conventional histology. Additionally, *in vitro* monitoring of the calcification processes of the tissue matrix is envisioned. Future studies are needed to evaluate the pericardium anisotropy in relation to the GA-fixation process. Regarding the calcification, hydroxyapatite precipitation of the solution can be potentially distinguished from collagen intercalation.

Author Statement

Special acknowledgement is entitled to the excellent technical assistance of Maria Feilmeier. Images were acquired in the Biopolis Dresden Imaging Platform at BIOTEC/CRTD Light Microscopy Facility and the MTZ-Core Facility Cellular Imaging (CFCI) with the assistance of Dr. Hella Hartmann, Ellen Geibelt, Dr. Anja Walther and Silke Tulok. JG and FS acknowledge support by the Free State of Saxony and the European Union EFRE (SAB project “OGD-LOC”).

References

[1] Rajput, F.A. and R. Zeltser, Aortic Valve Replacement, in StatPearls. 2025: Treasure Island (FL).
[2] Beckmann, A., et al., Thorac Cardiovasc Surg, 2024. 72(5): p. 329-345.
[3] Kueri, S., et al., Deutsches Arzteblatt International, 2019. 116(25): p. 423.
[4] Grebenik, E.A., et al., Biotechnol J, 2020. 15(8): p. e1900334.
[5] Bismee, N.N., et al., J Cardiovasc Dev Dis, 2024. 11(12).
[6] Kostyunin, A.E., et al., J Am Heart Assoc, 2020. 9(19): p. e018506.
[7] Velho, T.R., et al., Braz J Cardiovasc Surg, 2022. 37(2): p. 239-250.
[8] Dittfeld, C., et al., Biomater Adv, 2023. 147: p. 213328.
[9] Golomb, G. and D. Wagner, Biomaterials, 1991. 12(4): p. 397-405.
[10] Oyane, A., et al., Journal of Biomedical Materials Research Part A, 2003. 65a(2): p. 188-195.
[11] Kiesendahl, N., et al., Ann Biomed Eng, 2021. 49(2): p. 885.
[12] Bouma, B.E., et al., Nat Rev Methods Primers, 2022. 2.
[13] Kawasaki, M., et al., J Am Coll Cardiol, 2006. 48(1): p. 81-8.
[14] Xu, C., et al., J Biomed Opt, 2008. 13(3): p. 034003.
[15] Mehanna, E., et al., Circ J, 2013. 77(9): p. 2334-40.
[16] Golde, J., et al., J Biomed Opt, 2018. 23(7). p. 1.
[17] Bezuidenhout, D., et al., Biotechnol Appl Biochem, 2009. 54(3): p. 133-40.
[18] Williams, D.F., et al., Frontiers in Cardiovascular Medicine, 2021. 8.
[19] Zioupos, P., J.C. Barbenel, and J. Fisher, Med Biol Eng Comput, 1992. 30(1): p. 76-82.
[20] Zioupos, P., J.C. Barbenel, and J. Fisher, J Biomed Mater Res, 1994. 28(1): p. 49-57.
[21] Dittfeld, C., et al., PLoS One, 2018. 13(12): p. e0208