

3D-Sectioning of Pericardium with the ROWIAK TissueSurgeon

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Introduction

Pericardium is used in different applications in tissue engineering. Therefore it is necessary to cut it into customized pieces. Mechanical sectioning with scalpels or blades has certain disadvantages: blades induce mechanical shear forces and at the cutting edge the tissue is squeezed. Blades are also a risk of contamination. The ROWIAK Tissue-Surgeon offers a new way to overcome these disadvantages: sectioning is performed contact-free with femtosecond-laser technology.

Material and Methods

Porcine pericardium pieces (2 x 2 cm) were fixed in glutaraldehyde for at least 24 h and washed in PBS afterwards. Samples were transferred to a high precision microscope slide (Schott Nexterion B) in PBS. The sample was fixed on the slide with a second microscope slide on top. For testing, cuboids were cut with an edge length of 300 x 300 μm and a height of 180 μm (maximum thickness of pericardium, calculated with OCT, Fig. 1a). Cuboids were removed with forceps and imaged with Zeiss Primostar in transmitted light.

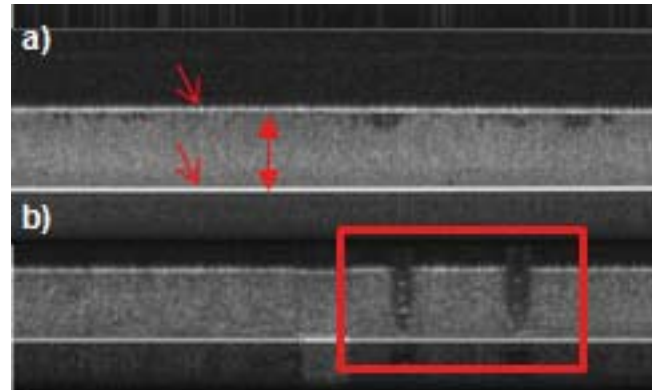


Fig. 1: OCT of porcine pericardium a) before and b) after cut. Upper and lower white line (arrows) indicate the microscope slides. The height of the tissue is 180 μm (double arrow). The successful section is highlighted by the red box (b).

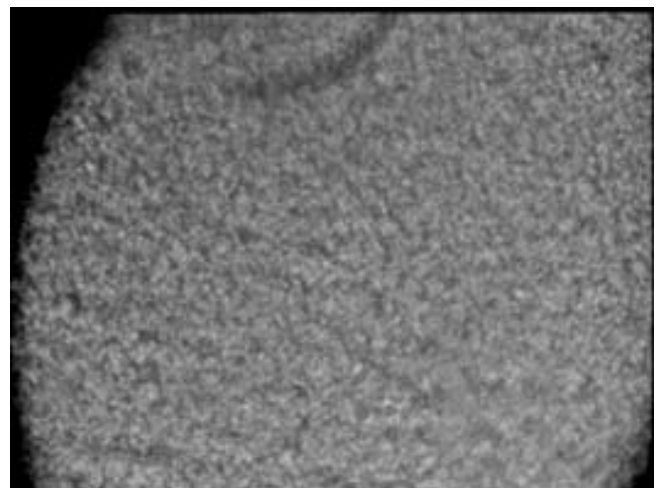


Fig. 2: Camera image of porcine pericardium. Camera is used to monitor the cutting process and to choose the area of sectioning in transparent tissues.

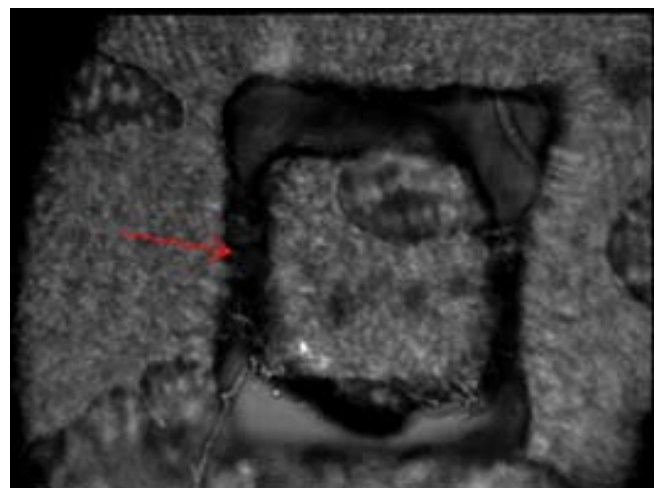


Fig. 3: Camera image of porcine pericardium of cutting a cuboid of 300 x 300 μm with a height of 180 μm . The red arrow points to the distinct separation

Results

3D sections can easily be performed with the ROWIAK TissueSurgeon. OCT-imaging shows distinct separation of the cut structure from the surrounding tissue (Fig 1 b). The integrated camera can be used to choose the area of interest (Fig. 2) and to observe of the cutting process. Camera imaging also shows distinction between the structure and the rest of the tissue (Fig. 3). The cut cuboid was easily removed by forceps under the dissecting microscope. Each step was documented in transmitted light with a microscope (Fig. 4 – 6). Cutting edges of the rectangular and the remaining pericardium are exact. The process of cutting one cuboids (300 x 300 x 180 µm) lasts around 50 seconds.

Conclusion

The ROWIAK TissueSurgeon offers a new approach to cut pericardium for tissue engineering without contamination and shear forces, that squeeze the cutting edges. Cut samples show distinct and exact cut edges. The demo system is limited to sizes of 13 x 13 mm. For larger samples, the software and sample stage can be adapted, which is part of our customized design based on customers requirements.

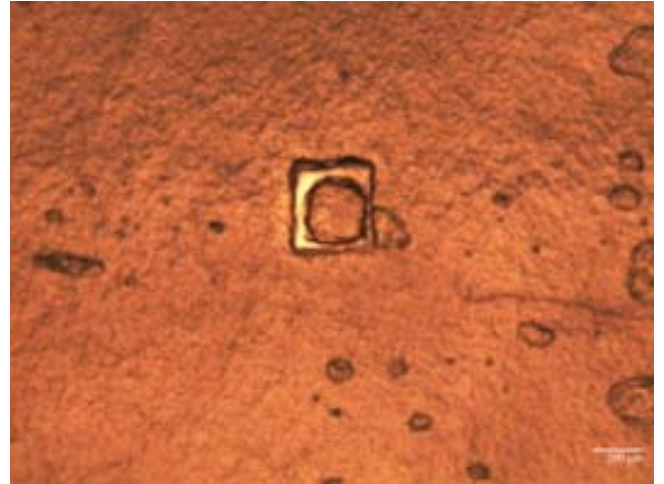


Fig. 4: Microscope image after cut with cuboid still in position

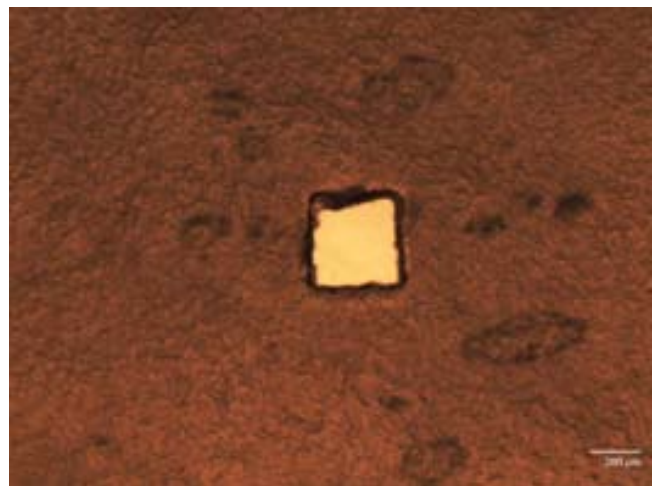


Fig. 5: Microscope image :Pericardium after removal of cuboid

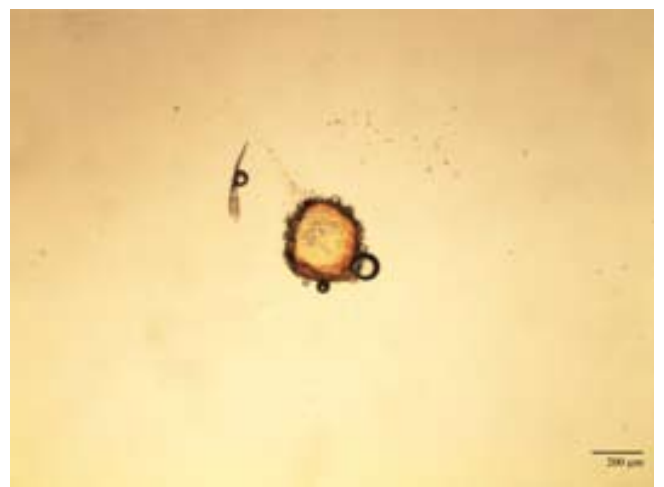


Fig. 6: Microscope image: Cuboid removed after cutting. Cuboid tends to constrict after removal due to the high content of elastic fibers