Sectioning Coronary Arteries Containing Metal-Stents with ROWIAK TissueSurgeon

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Introduction

In cardiovascular medicine insertion of metal stents into arteries is a frequently used method to support them. When seeking product approval from a regulatory body such as the FDA, the stent material must first be tested in a preclinical animal model. In order to determine product safety and efficacy, histology is required. Current histological methods employed to collect this data require stented vessels to be first embedded into resin or plastic and then sectioned thick using a diamond studded blade/wire or sectioned thin using a tungsten-carbide knife. The stent material, which is mostly manufactured from some form of metal, can create and be subjected to many artefacts during these mechanical contact methods of microtomy. These methods can also be quite costly due to the frequency of knife re-sharpening and the eventual replacement of both knives and diamond studded blades/wires as they become useless. Additionally, the collection of sections is far from easy, as both methods typically require multiple lengthy steps in order to achieve a mounted section ready for staining.

With the ROWIAK TissueSurgeon, we would like to present a new, non-contact laser-based process to prepare such sections, that is:

a) Time saving with higher throughput
b) Routine and easy to perform
c) Material saving compared to ground sections

Material and Methods

Coronary arteries with stents were embedded into Spurr's embedding medium. The resulting blocks were trimmed into tissue segments with a diamond saw and the cut surface was lightly polished by hand. The polished surface was then mounted on a glass microscope slide with a fast curing (one minute) cyanoacrylic based glue. Quality of mounting was controlled with Optical Coherence Tomography (OCT; Fig. 1), integrated with a laser cutting device (ROWIAK TissueSurgeon). Afterwards, the sample was cut at 10 µm thickness with section success again monitored via OCT (Fig. 2). Staining (Sanderson's Rapid Stain) and coverslipping was prepared with similar protocols used for thick/ground and thin section methods. Remaining blocks were polished, re-mounted and re-processed.

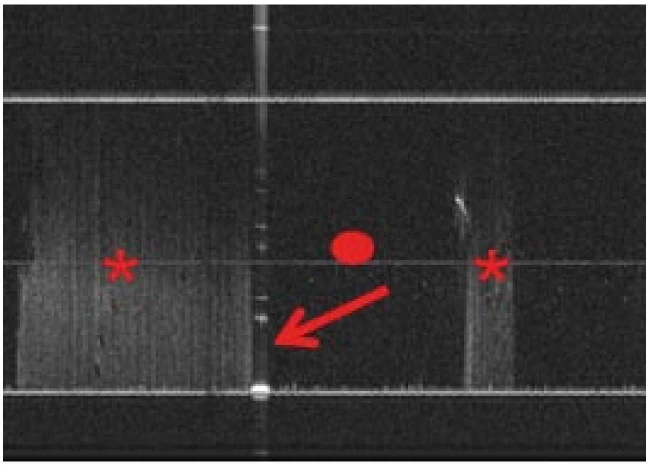

Fig. 1: OCT of coronary artery containing metal stent (arrow) located at the endothelium of the artery. Surrounding tissue of the artery is highlighted by asterisks, lumen of the artery by a dot.

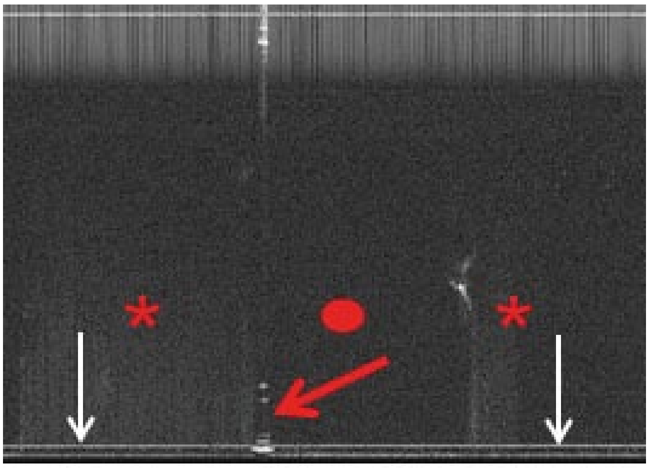

Fig. 2: OCT of coronary artery containing metal stent (arrow) located at the endothelium of the artery (as in Fig. 1) after cutting with the TissueSurgeon. Successful section is indicated by a white line (white arrows)

Fig. 3: Histology of artery containing stent after sectioning with the TissueSurgeon. In the gaps, the stent struts were located (arrow)

Results

Sections of Spurr's embedded arteries with implanted stents can easily be prepared with the ROWIAK TissueSurgeon. Histological quality is comparable to sections cut thin with a rotary microtome (Fig. 3). The stent material is not pushed or removed from its origin, creating artifacts but just removed uncut, leaving the tissue implant interface (Fig 4). Details of endothelium and artery are clearly visible at higher magnifications (Fig. 5). Sample throughput yields 10-12 samples ready for staining in an hour. Loss of sample material compared to thin section methods can be neglected as 10-12 samples sectioned at 10-15 µm each take away a measured thickness of no more than 180 µm from the sample block. Loss of sample material compared to ground section methods can be neglected as semi-serial sections are attainable without specimen loss attributed to diamond cutting and multi-layer grinding/polishing.

The TissueSurgeon was operated by a Senior Histologist, with prior experience utilizing conventional methods to perform the same task, using the TissueSurgeon for the first time during this proof of concept. An introduction of 30 minutes was more than enough time to become familiar with the software and develop a routine to produce at high throughput.

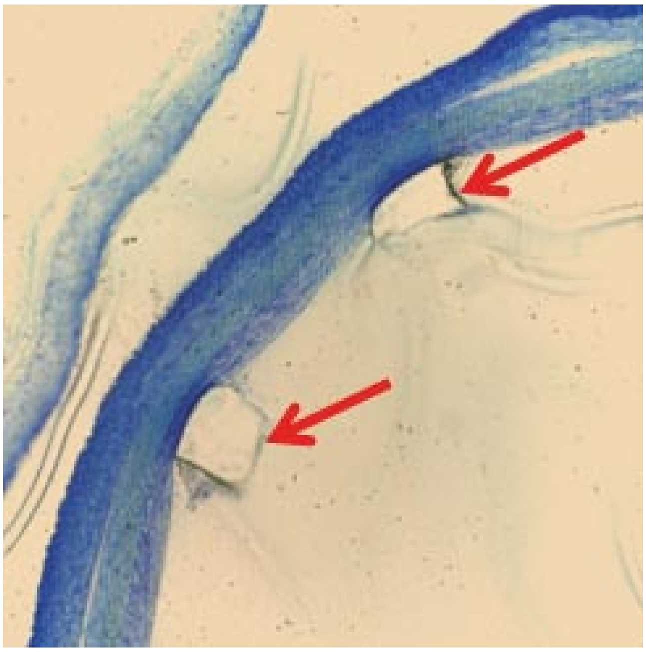

Fig. 4: Two locations of stent struts (arrow) covered with endothelium

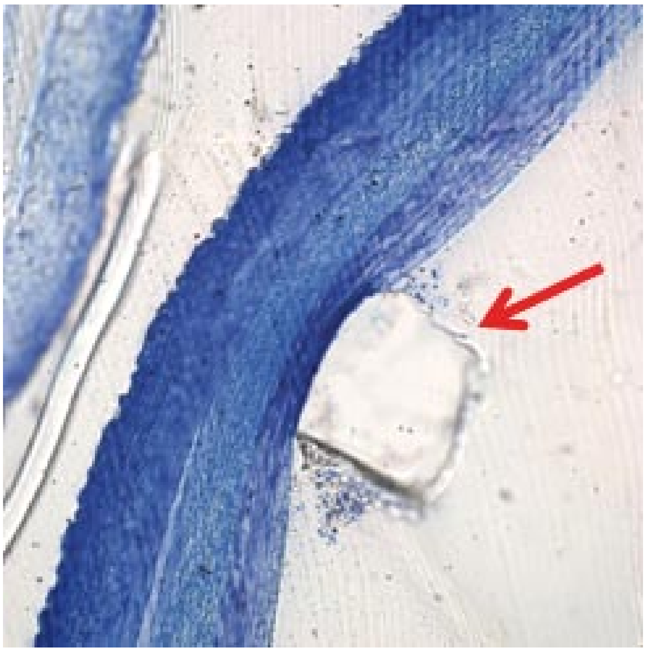

Fig. 5: Detail of stent strut (arrow) and details of artery wall

Conclusion

The ROWIAK TissueSurgeon offers a new non-contact method of microtomy to prepare sections of resin embedded vessels containing metallic stents. This laser based technology creates sections for histology in similar quality as compared to conventional sectioning methods utilizing diamond studded blades/wires and tungsten-carbide knives. Artifacts, created by contact methods of microtomy are eliminated by the use of non-contact laser-based cutting. The real benefit is in high throughput compared to conventional sectioning methods: the TissueSurgeon doubles the amount of samples ready for staining per hour (10-12 samples), compared to thin section methods and the process is exponentially faster as compared to thick/ground section methods. High throughput is generated by the fact that there are minimal steps of preparation and enhanced reproducibility of current steps. Reproducibility is achieved with the introduction of a new fast curing cyanoacrylic based ROWIAK glue. The rest of the work is done via a software interface that is fast and user friendly.