Published by Buehler, a division of Illinois Tool Works

Volume 1, Issue 5

SUM NOTES

Preparation of Intravascular Stent Thin Sections

Introduction

Coronary stents are metallic frames expanded with a balloon catheter inside an artery that has closed or appears likely to close. Coronary stents restore sufficient blood flow to the heart muscle by propping open the artery.

The inventor of the coronary stent was Charles Dotter, who developed his first stent in 1969. Charles Dotter continued to refine and develop his design and in 1983, together with Andrew Craig, invented an expandable stent made out of nitinol, a material that is frequently used in stents today

While the use of stents has become more commonplace, developing a better understanding of the interaction between the human body and stents remains an important clinical topic. The study of different stent designs, materials, surface coatings, and adjunctive drug treatment requires detailed histological and immunohistochemical analyses of the stented vessel, especially at the tissue metal interface with the struts in situ.

Proper preparation techniques will enhance understanding of the cellular response to clinical stenting, especially at the tissue stent interface. In addition, it will allow close evaluation of the expansion characteristics. Such observations may even lead to the development of improved stent designs.

Preparation Procedure

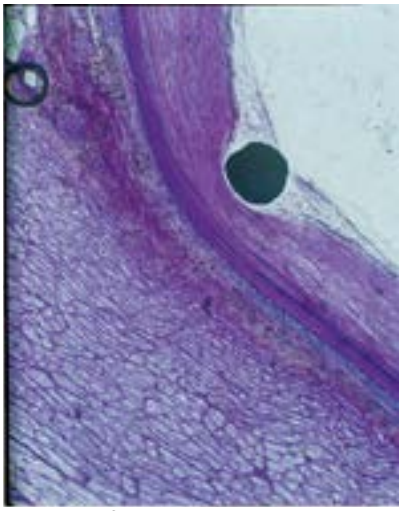
1. Remove the stented arteries, flush with saline, and immersion fix in formal saline for two hours.

2. Embed the stented arteries prior to sectioning. Select a mounting compound that will minimize shrinkage and will not soften during the sectioning procedure. A low viscosity epoxy, such as EpoThin®, is an excellent choice.

   Measure the resin and hardener according to the directions on the product label. Blend the ingredients thoroughly, but gently to avoid excessive formation of air bubbles. Allow the mixture to sit for a few minutes before using, to allow any remaining entrapped air to rise to the top.

   Place the specimen in a mold and vacuum impregnate the specimen with the epoxy. Pour sufficient epoxy to completely cover the specimen.

3. Prior to sectioning with a precision saw, grind the end surface of the mount with 600 (P1200) grit and 1200 (P2500) grit CarbiMet® SiC Abrasive Paper using a MetaServ® 2000 Grinder-Polisher or similar polisher.



Thin section of a stented artery showing the tissue stent interface.

4. Adhere the ground surface to a slide using a contact adhesive. Perspex slides provide greater adhesion of the embedded sections than glass slides. However, pregrinding a glass slide with 600 (P1200) grit CarbiMet® SiC Abrasive Paper will improve the adhesion properties.

5. Select a blade to section the material that will minimize kerf loss (the amount of material lost or removed from the specimen due to thickness of the blade passing through the specimen). Note: the flanges (blade supports) should be selected to provide maximum support when using a thin blade to prevent bending or torquing during sectioning.

   The IsoCut® Wafering Blade with bonded cubic boron nitride enables between 28 and 30 sections per 15mm stent to be reproducibly obtained.

6. Mount the encapsulated specimen onto the chuck of an IsoMet® Precision Saw. Align the micrometer to remove a 50 to 100µm section from the slide end of the specimen.

7. Prepare the block surface for the next section by repeating steps 3 and 4. Repeat the process to give consecutive sections

affixed to slides.

8. Grind each section to the required thickness of 10–20µm using a series of CarbiMet® Silicon Carbide Abrasive Papers. The SiC fixed abrasive is used to prevent embedding abrasives in the tissue part of the specimen.

   Start with 320grit (P400) and then proceed to 360 (P500), 400 (P800), 600 (P1200) and 1200 (P2500) grit. Work carefully to avoid removing material too rapidly or unevenly. Between each step verify that a uniform scratch pattern has been achieved prior to moving on to the next abrasive paper.

9. Polish using 3µm UltraPrep™ Type A Diamond lapping film prior to conducting the final observations.

Automation/ Special Fixtures

Grinding and polishing thin sections by hand requires a great deal of expertise and time. Grinding by hand also tends to favor one or the other side of the thin section, eventually making one side thinner than the other side.

An uneven surface and thickness will provide a less than optimal histochemical analysis. To prepare specimens for histological and immunohistochemical staining, the specimens have to be ground to an even thickness of 5µm.

To minimize the problems mentioned above, when a specimen is to be prepared by hand, use a holder with fixed stops. For example, the Histolic Precision Grinding Fixture (60-8087) has carbide stops that prevent the specimen from being over ground and helps in obtaining a specimen of uniform and of desired thickness. The fixture is designed for slides that are 27 x 46mm in size.

The portable BioThin™ Thin Sectioning Device (38-2100) has been used with great success to achieve5µm sections. The fully enclosed system uses ball bearings to control the amount of applied force and position of the slide. For the final thinning, prior to staining, it is recommended that the 3µm UltraPrep™ Type A Diamond Lapping Film is used.

The grinding process can be automated on most semi-automated polishers by using a Petrographic/ Histolic Thin Section Slide Holder (69-1584) and single force application. The slide containing the sectioned specimen is attached to the slide holder using double-sided tape. The individual slide holders are then placed into the Single Force Slide Holder. Apply 3 to 5 psi depending on the removal rate required. Evaluate the specimens at least every 2 minutes until the best procedure has been established.

Bibliography

M Bellis, "Coronary Stents" Inventors – Cardiac Pacemaker/ Electrocardiology.http://inventors. about.com/library/inventors/blcardiac.htm (last viewed Oct 15, 2004). M. Malik, J Gunn, CM Holt, L Shepherd, SE Francis, CMH Newman, DC Crossman, DC Cumberland, "Intravascular stents: a new technique for tissue processing for histology, immunohistochemistry, and transmission electron microscopy" Heart 1998; 80:509-516.

Equipment*
Cast N' Vac Castable Vaccum System
IsoMet® Family of Linear Precision Saws
MataServ® Grinder-Polisher or other Grinder-Polisher

Consumables*
IsoCut® CBN Wafering Blade
EpoThin® Low Viscosity Epoxy
CarbiMet® Abrasive Discs
UltraPrep™ Diamond Lapping Film

**For a complete listing of Buehler Equipment and Consumables, please refer to Buehler's Equipment Buyer's Guide and Buehler's Consumables Buyer's Guide*

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