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RESIN HISTOLOGY METHODS FOR STENTED ARTERIES

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Comparison of Processing and Sectioning Methodologies for Arteries Containing Metallic Stents

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ABREVIATIONS:

AES	3'aminopropyltriethoxysaline
BMA	butyl methacrylate
DAB	3,3' diaminobenzidine
FITC	fluorescein isothiocyanate
GMA	glycol methacrylate
H&E	hematoxylin & eosin
IEM	immuno – electron microscopy
ISR	in-stent restenosis
MMA	methyl methacrylate
NBF	neutral buffered formalin
NBT	nitroblue tetrazolium
PBS	phosphate buffered saline
PCNA	proliferating cell nuclear antigen
RT	room temperature
SG	saw grind
SMA	smooth muscle α-actin
TC	tungsten carbide
TEM	transmission electron microscopy

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Abstract

The histological study of arteries with implanted metallic scaffolding devices, known as stents, remains a technical challenge. Given that the arterial response to stent implantation can sometimes lead to adverse outcomes, including the re-accumulation of tissue mass within the stent (or in-stent restenosis), overcoming these technical challenges is a priority for the advancement of research and development in this important clinical field. Essentially, the task is to section the stent-tissue interface with the least amount of disruption of tissue and cellular morphology. Although many methacrylate resin methodologies are successfully applied towards the study of endovascular stents by a variety of research laboratories, the exact formulations, as well as subsequent processing and sectioning methodology remain largely coveted. In this paper we describe in detail a methyl methacrylate resin embedding methodology that can successfully be employed towards tungsten carbide blade, as well as saw and grinding sectioning methods and transmission electron microscopy. In addition, we present a comparison of the two sectioning methodologies in terms of their effectiveness with regards to morphological, histochemical and immunohistochemical analyses. This manuscript contains online supplemental material at http://www.jhc.org. Please visit this article online to view these materials.

Key words: stent, artery, restenosis, methyl methacrylate, resin, immunohistochemistry

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Introduction

The past decade has witnessed a marked increase in the use of stents for the treatment of obstructive vascular disease (Froeschl et al. 2004). Stents are metallic scaffolds that are inserted on a balloon catheter into a blood vessel, and with balloon expansion are deployed against the vessel wall to restore patency (**Supplemental Figure 1**). Renarrowing of stents, or in-stent restenosis (ISR) is a major drawback of this medical technology, occurring in approximately 5-35% of cases (Moses et al. 2003; Sketch et al. 2005). Although coating of stents with polymers that elute a drug that may help prevent cell growth and/or inflammation has somewhat attenuated this problem, it is estimated that in North America alone 100,000 patients per year will continue to develop ISR (Chu et al. 2005). Therefore, attenuating the frequency of ISR remains an important goal of vascular interventional research. Unfortunately, our understanding of the arterial reaction to stent implantation is incomplete - primarily due to technical limitations in the histological study of stented vascular tissue (Froeschl et al. 2004). Conventional paraffin embedding and sectioning procedures have demonstrated limitations with regards to their application towards the histological study of the vascular effects of endovascular stent implantation. In most cases these procedures require the complete removal of the metal stent prior to tissue processing thereby disrupting normal vascular architecture and in particular the stent-tissue interface. In order to accurately observe the vascular response it is imperative that the stent-tissue interface is sectioned with the least amount of disruption of tissue and cellular morphology. To this end it is now common practice to employ methacrylate resins as the embedding media of choice (Chen et al. 2004; Ma et al. 2004).

Methacrylate resins are successfully utilized as embedding media for hard biological tissues such as undecalcified bone samples and these methods have recently been adapted or further modified

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to suit the study of stented vessels (Theuns et al. 1993). Currently glycol methacrylate (GMA), methyl methacrylate (MMA) and a combination of MMA & n-butyl methacrylate (BMA) are the most widely used resins for the histological processing of endovascular stented material (Brasen et al. 2001; Malik et al. 1998; Rogers et al. 1996; van Beusekom et al. 1996). Although many of these methacrylate resin methodologies are employed by a variety of vascular research laboratories, the exact formulations, as well as the processing and sectioning methodologies have largely remained coveted. In this paper we describe in detail an MMA resin embedding methodology which can be successfully employed towards both tungsten carbide (TC) blade as well as saw and grinding (SG) sectioning methods. In addition, we present a comparative review of the two sectioning methodologies in terms of their effectiveness with regards to morphological, histochemical, immunohistochemical and transmission electron microscopy (TEM) analyses. It is our intention that these resin embedding and sectioning methodologies will serve as a template for other laboratories embarking on the study of intravascular stent pathology.

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Materials and Methods

Stented human coronary arteries from patients were obtained at necropsy and processed as follows.

Tissue Processing

Coronary arteries were removed from the heart , fixed in 10% neutral buffered formalin (NBF) for greater than 24 hours, decalcified in 10% formic acid and subjected to radiography in order to localize the stent(s). Decalcification of the entire artery is routinely performed at The Ottawa Hospital in order to accurately assess adjacent atherosclerotic plaques at either end of the stent. The isolated stented vascular segments were then transferred to 70% ethanol before being processed and embedded in MMA resin (for schedule details see **Supplemental Table 1**) (Erben 1997). The following solutions were used to make the resin mixture: Solution I [60 ml MMA (Marivac Inc, Montreal, QC, Canada), 35 ml BMA (Marivac), 5 ml methylbenzoate, and 1.2 ml polyethylene glycol 400], Solution II (100 ml of solution I, 0.4 g benzoyl peroxide), Solution III (100 ml of solution I, 0.8 gm benzoyl peroxide) and a polymerization resin mixture (400 µl of N,N-dimethyl-P- toluidine added to 100 ml of solution III and stirred for a few minutes before use). All resin solutions were kept at 4ºC and stirred for at least 1 hour before use.

Infiltrated stented segments were carefully placed in an upright position within 20 ml polypropylene vials (Leica) which were completely filled with the polymerization mixture in order to exclude air, then tightly capped and polymerized at -18° to -20°C. The presence of oxygen within the embedding vials can ultimately cause bubble formation thereby inhibiting adequate polymerization and adversely affecting subsequent sectioning technique. This can however be prevented by either

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bubbling nitrogen gas into the tissue embedded, resin filled vials or by embedding the tissue segments into vials containing prepolymerized resin bases or plateaus (Erben 1997).

Depending upon specimen size, other suitable molds include glass vials (Fisher Scientific) polypropylene microcentrifuge tubes (Fisher Scientific), Eppendorf tubes (Fisher Scientific) or Beem capsules (Marivac). Following adequate polymerization, the specimens are removed by either cutting the ends off the polypropylene vials or alternatively breaking the glass vials and pushing the specimens out. The polymerized blocks were then shaped and trimmed of excess plastic with a grinder and sandpaper prior to sectioning.

Rotary Microtome and Tungsten Carbide Blade Method of Tissue Sectioning

Tissue sections of a 4-5 µm thickness were generated on an automated Leica RM2255 rotary microtome utilizing a D profile tungsten carbide (TC) blade (Leica Instuments GmbH, NuBloch, Germany). As MMA resin is hydrophobic, sectioning was facilitated by moistening both the specimen block and knife edge with 30% methanol. In a 42ºC waterbath, cut sections were picked up with forceps and first floated onto the surface of a solution of 30% methanol for stretching and then transferred onto the surface of a 0.1% aqueous Elmer's glue solution to promote adherence of the section to the slide (**Figure 1A**). The sections were then picked up on 3'aminopropyltriethoxysaline (AES) treated slides (Sigma-Aldrich; Oakville, Ontario, Canada), stretched again with chloroform vapors, covered with a thin plastic film and gently flattened with a rubber roller (**Figure 1B**). Finally the slides were carefully stacked, pressed together with a spring clamp and dried in an oven at 42ºC for a period of 2 days (**Figure 1C**).

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Saw Grinding Method of Tissue Sectioning

A Buehler IsoMet 5000 high speed precision saw (Buehler; Dusseldorf, Germany) was used to section the stented specimens at a cutting speed of 3200 rpm together with a feed advance of 12mm/min. Continual water cooling was employed throughout sectioning and a cooling agent (Isomet Plus lubricant, at a ratio of 9:1) was added to reduce heat caused by friction. The surfaces of the resin blocks were prepared for sectioning by grinding on a Metaserve 2000 grinder (Buehler) using fine 600 grit water paper in order to remove irregular surfaces in the block. Clean, dry blocks were glued onto polysine slides using cyanoacrylate super glue by placing a small drop of glue on the block surface and holding it firmly in position on the slide for 20 seconds (**Figure 1D**). Care must be taken to avoid irregular pressure as this will result in the formation of bubbles underneath the section. Slides were secured in the slide holder of the Isomet saw and 100 µm sections were cut (**Figure 1E**). A wafering diamond blade (12.7mm × 0.4mm; Buehler) was used for this purpose. A dressing stone was used to maintain a clean blade edge. Thick sections were ground on the Metaserve 2000 grinder (Buehler) using 2 grades of water paper (Buehler) (**Figure 1F**). Coarse 600 grit paper was used to obtain the required thickness, followed by a polishing with 1200 grit paper to remove any scratches. A fine residue of sanding grains was sometimes noted and attempts to remove the residue using isotonic baths, mild detergents or polishing were unsuccessful. A final thickness of 8-10 µm was achieved.

Histochemistry

After undergoing deplasticization at room temperature (RT), tissue specimens sectioned using the TC blade method were stained with Gill 3 hematoxylin (Fisher) and eosin (H&E), Movat pentachrome, Masson trichrome, Azan and Verhoeff's Van Gieson dyes according to conventional

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methodologies (Bancroft et al 2001). For deplasticization the sections were brought through solutions of 2-methoxyethylacetate for 20 minutes thrice, acetone for 5 minutes twice, and distilled water for 5 minutes twice.

In our experience deplasticization of SG sections was often unsuccessful due to the reaction of the solvents with the cyanoacrylate glue used to affix the sections onto the slides, thereby resulting in the sections becoming detached from the slides. A limited number of stains were made possible without prior removal of the resin and these included Mayer's H&E and Verhoeff's elastin . For H&E staining of SG sections air dried slides were placed on a hot plate at 60°C and covered with Mayer's hematoxylin (Sigma-Aldrich; Steinheim, Germany) for 12 minutes taking care not to let the slides dry out. Slides were then rinsed and blued in running tap water for 30 minutes before further staining with Eosin/Phloxine (1:1) on a hot plate at 60°C for 1 minute, rinsing in water and finally mounting in Glycergel (DakoCytomation; Glostrop, Denmark). For Verhoeff's elastin staining, slides were immersed in Verhoeff's solution (20ml alcoholic hematoxylin, 8ml 10% Ferric chloride, 8ml Lugols Iodine) for 20 minutes at RT, rinsed in tap water and mounted in Glycergel (Dakocytomation) (**Figure 2**).

Immunohistochemistry

Deplasticized TC sections were permeabilized in 1% Tween 20-phosphate buffered saline (PBS) for 30 minutes followed by incubation with primary antibodies diluted in 1% BSA at RT for 1 hour. The following primary antibodies were used: Factor VIII (DakoCytomation, 1:50), smooth muscle α-actin (SMA; 1:100, RDI, Concord, MA, USA), vimentin (DakoCytomation, 1:100), HAM 56 (DakoCytomation, 1:100), and proliferating cell nuclear antigen (PCNA, Santa Cruz, 1:100).

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Following two washes with 1% Tween 20-PBS for 5 minutes tissue sections were incubated with a secondary antibody (DakoCytomation; EnVision K4000, HRP labeled Polymer) for 30 minutes at RT. The standard peroxidase enzyme substrate 3,3'-diaminobenzidine (DAB, DakoCytomation) was applied as the chromogen. Slides were dehydrated in graded alcohol, cleared in xylene and mounted in entellan (Sigma-Aldrich). PCNA immunolabeling was performed in a different manner, as tissue sections were incubated with a fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG antibody (1:100, Vector Laboratories Inc. Burlingame, CA) for 30 minutes and then mounted in 50% glycerol in PBS. Photomicrographs were obtained using a fluorescence microscope (Olympus, Melville, NY, USA). The same slide was then washed with PBS, subjected to Masson's trichrome staining and repeat photomicrographs were obtained (**Figure 3**).

Combination of immunofluorescence and histochemistry in same tissue section

Multi-label immunofluorescence detection is an invaluable tool for both research and diagnostic endeavours, dramatically advancing the study of cell- and tissue-specific expression of many proteins (antigens). In the tissue section, however, photographed immunofluorescence is difficult to interpret with respect to the morphological relationships among various cells and their environments. Here we are the first to report a technique for presenting both immunofluorescence and histochemistry on the same section of MMA embedded human coronary arterial stent tissue. Briefly, the deplasticized TC sections were incubated in 3% skim milk for 1 hour at RT followed by incubation with mixed primary antibodies at 4°C overnight. The following mixed primary antibodies were employed: rabbit anti-PCNA and mouse anti-SMA (DAKO, 1:100), rabbit anti-PCNA and mouse anti-macrophage (CD68, DAKO, 1:100). Following triplicate washes in PBS the sections were incubated with mixed Texas red anti-mouse IgG (1:100, Vector Laboratories, Inc. Burlingame,

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CA) and fluorescent anti-rabbit IgG (1:100, Vector Laboratories Inc. Burlingame, CA) secondary antibodies for 30 minutes at RT. Three washes with PBS were again performed before mounting in 50% glycerol in PBS. Photomicrographs were obtained using a fluorescence microscope (Olympus, Melville, NY, USA). The same slide was then washed with PBS, subjected to Masson's trichrome, H&E staining and repeat photomicrographs were obtained, respectively (**Figure 4**).

Immunohistochemical staining of SG sections was performed without prior deplasticization. SMA immunolabeling was done with overnight incubation of the primary antibody (1:100) at 4°C. Following 2 washes in 0.1% Tween 20-PBS, Alkaline Phosphatase-conjugated (EnVision, DakoCytomation) anti-mouse secondary antibody was applied for 30 minutes at RT, and the interaction was visualised with nitroblue tetrazolium (NBT; Sigma-Aldrich) as the chromogen. Sections were mounted with Glycergel without prior dehydration through graded alcohol.

Transmission Electron Microscopy

Two 10% NBF fixed human coronary artery specimens were processed in the outlined MMA resin mixture for both routine TEM and immuno-electron microscopy (IEM) analyses. Post fixation with 1% osmium tetroxide was omitted for the specimens designated for IEM. Thin tissue sections (60-90 nm) were cut on a Reichert Ultracut E ultramicrotome using a diamond knife (Diatome, Hatfield, PA, USA) and mounted on formvar coated nickel grids (Marivac). Tissue sections for routine TEM were counterstained with 3% aqueous uranyl acetate (Marivac) for 30 minutes and Reynolds lead citrate (Marivac) for 5 minutes. In contrast, tissue sections for IEM analysis were placed onto drops of 1% BSA in PBS for 30 minutes at RT and without rinsing the sections were placed onto drops of the primary antibody, polyclonal anti-SMA (DakoCytomation, 1:100 diluted in 0.1% BSA-PBS),

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before being incubated overnight at 4ºC. After rinsing in PBS the sections were placed onto drops of a secondary 15 nm gold conjugated goat anti-rabbit IgG (EY Laboratories,San Mateo,CA,USA, 1:100) for 1 hour at RT. Gentle rinsing in distilled water was followed with counterstaining in aqueous 3% uranyl acetate and Reynolds lead citrate. The stained sections were then examined by TEM (JEOL 1230, JEOL, Peabody, MA, USA) equipped with software from Advanced Microscopy Techniques (AMT, Danvers, A, USA).

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Results

Sectioning

Thin tissue sections were consistently produced with a TC knife. The main concern with the TC knife method was the potential for blade damage and resultant scoring of tissue sections. In contrast, production of SG sections proved to be more laborious and technically challenging. As the SG sections are considerably thicker, specimen depletion was also a concern. Typically, only 1-2 SG sections were produced per block.

Morphology

Although both sectioning methods yielded sections of acceptable morphological integrity, fine histological detail was more readily observed in the thinner TC sections (**Figure 2**). Section thickness proved variable with the SG method due to uneven section surfaces as compared to consistently uniform thickness with the TC method. Sectioning artifacts such as scoring and folding were not present in SG sections but occurred with a noticeable frequency in TC sections. Stent struts consistently remained *in situ* for SG sections but were often displaced or lost within TC sections (**Figure 2**).

Histochemical staining

The range of histochemical staining methods applicable to SG sections was limited due to the incompatibility of the deplasticization solvent and the glue utilized to fix the tissue sections to the slides. Staining was therefore accomplished without deplasticization and limited to H&E and Verhoeff's elastin stains (**Figure 2**). In contrast, TC sections could be deplasticized and

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accommodated a broader range of histochemical stains such as H&E, Movat, Azan, Masson, Orcein/Resorcin Fuchsin and Verhoeff's Van Gieson (**Figure 2**).

Immunohistochemical Labeling

The range of immunocytochemical stains applicable to SG sections was limited to SMA for reasons previously described. TC sections were deplasticized and stained for specific markers such as SMA, vWF, HAM56 (**Figure 3**). Moreover, we devised a novel method of combining fluorescent immunolabeling and histochemical staining on the same slide. For example, we immunolabeled specimens with a fluorescently tagged antibody to PCNA, obtained a fluorescent photomicrograph, and then performed a Masson's trichrome stain on the same section before repeating light photomicrographs of the same tissue section (**Figure 4**). This combined approach allowed us to specifically localize proliferating cells in relation to various components of the extracellular matrix of these lesions.

TEM & Gold Labeling

The MMA resin proved conducive for the performance of both routine TEM and immunogold analyses. For example, 15 nm gold-labeled anti-SMA antibody was successfully used to immunolabel smooth muscle cells (**Figure 3**).

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Discussion

With the escalating global use of stents to treat patients with obstructive coronary artery disease the problem of ISR is of paramount importance. Although drug coated stents may reduce the frequency of ISR, the overall incidence of ISR is still estimated to be approximately 9%, and cost may prevent the widespread use of these bioprostheses (Waksman, R. 2005). Currently, there is much interest in better understanding the vascular response to stent implantation, as well as identifying *bona fide* therapeutic targets to prevent ISR. Unfortunately, not all laboratories are adequately equipped or have sufficient experience to engage in the intricacies of sectioning, staining and immunolabeling vascular tissues that contain metallic bioprostheses. In this manuscript we review methodologies and outcomes of two sectioning methods for resin embedded arteries that contain metallic stents.

The SG method consistently produces intact tissue sections without displacement of stent struts nor scoring or folding of tissue. Moreover, it is superior to the TC method when stents made of more durable alloys, such as nitinol, are encountered or when longitudinal sections of stented arteries are required. Nonetheless, there are important limitations to note about the SG method. First, there is significant tissue depletion (kerf loss) with the SG technique, as only one or two sections can be produced per block. Second, because the minimum thickness of sections produced with SG is 8-10 μm, the quality of morphological studies may be inferior to the TC approach. Third, to produce multiple SG sections is laborious and certainly more technically challenging than producing TC sections. Fourth, the initial equipment setup costs for the SG method is considerably more expensive than that necessary to perform the TC method (refer to **Supplemental Table 2**).

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The TC method consistently yields thin serial sections that are suitable for both histochemical and immunohistochemical staining of de-plasticized sections (Figure 2). Although occasional scoring and folding together with stent strut displacement does occur in TC sectioning, in our experience this method clearly provides superior morphological and immunolabeling detail at the tissue-stent interface. For example, using this technique we have carefully examined stented vessels from animal arteries and determined the efficacy of novel anti-inflammatory therapies that reduce stent neointima formation (Chen et al. 2004; Ma et al. 2004). This method has also provided accurate information concerning the status of human coronary stents for both forensic and hospital autopsies performed over the past 8 years at TOH. Of note, others have used the SG technique utilizing Technovit 4000 glue (Heraeus-Kulzer, Friedrichsdorf,Germany) to adhere tissue sections onto slides and have obtained seemingly very acceptable immunolabeling results (Brasen et al. 2001; Rammelt et al. 2004) This has so far proven unsuccessful in our hands.

In summary, the described MMA embedding protocol adequately accommodates both the TC and SG methods of sectioning. Although either method of sectioning can be effectively utilized to evaluate the histopathological features of resin embedded vascular stents, the TC method allows thinner sections to be used, a wider range of histochemical staining and immunolabeling procedures to be readily performed. Future studies of the vascular response to a variety of current and newer stent materials (e.g., magnesium biodegradable stent) will provide important clues to the pathogenesis of ISR and may allow the formulation of a durable scaffolding device for the growing number of patients that require percutaneous revascularization.

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Figure legends

Figure 1: Equipment and procedure for cutting TC (A-C) and SG (D-F) sections. A: 4-5 µm tissue sections were cut on an automated Leica RM2255 rotary microtome utilizing a D profile TC blade. In a 42ºC waterbath, 0.1% aqueous Elmer's glue solution and 30% methanol were used for floating and promoting adherence of the sections to slides. B: The sections were picked up on AES treated slides, stretched, covered with a thin plastic film and gently flattened with a rubber roller. C: Stacked slides were pressed together with a spring clamp. D: Clean, dry blocks were glued onto polysine slides. E: Slides with blocks were secured in the slide holder of the Isomet saw (Buehler). 100 µm sections were cut with a wafering diamond blade (12.7mm × 0.4mm; Buehler). F: Thick sections were ground on the Metaserve 2000 grinder (Buehler) using 2 grades of water paper. Bar=20mm.

Figure 2: Histochemical staining. A & B: H&E stain (TC sections); C & D: H&E-Phloxine stain (Undeplasticized SG sections); E: Movat Pentachrome stain (TC section); F: Azan stain (TC section); G: Verhoeff Van Gieson elastin stain (TC section); H: Verhoeff Van Gieson elastin stain (Undeplasticized SG section). Bar=200 µm.

Figure 3: Immunohistochemistry was performed by applying various antibodies onto deplasticized TC sections and undeplasticized SG sections. A: HAM56 (TC section); B: vWF (TC section); C: SMA (TC section); D: SMA (SG section); E: Routine TEM (TC section); F: immunogold analysis with 15nm gold-labeled anti-SMA (TC section). Anti-SMA labeled dense bodies are indicated with arrows. Arrow heads define the border of the cell membrane. Bar=200 µm except where otherwise specified.

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Figure 4: Combination of immunofluorescence and histochemistry. The double immunofluorescence was performed with rabbit anti-PCNA (green) and mouse anti-SMA (red) antibodies (A), rabbit anti-PCNA (green) and mouse anti-macrophage (CD68, red) antibodies (C), respectively. After obtaining a photomicrograph, the same section was stained with Masson's trichrome (B), and H&E (D), respectively. Both PCNA- and SMA-immunopositive cells are indicated by arrows in panel (A), and panel (B). The CD68-immunopositive, PCNA-immunonegative cells are indicated by arrow heads in panel (C), and panel (D). A single PCNA-immunopositive, CD68-immunonegative cell is indicated by an arrow in panel (C), and panel (D). The inserted photos in the bottom left corner of panels B and D are the same sections shown at low power. The red stars indicate the former location / displaced of stent struts. Bar = 20 µm.

Supplemental Figure 1: A: Equipment used to deploy a stent. B, C & D: Metallic stent mounted on balloon catheter in undeflated (B) and inflated (C) state, as well as after being removed in fully expanded form (D).

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Figure 1

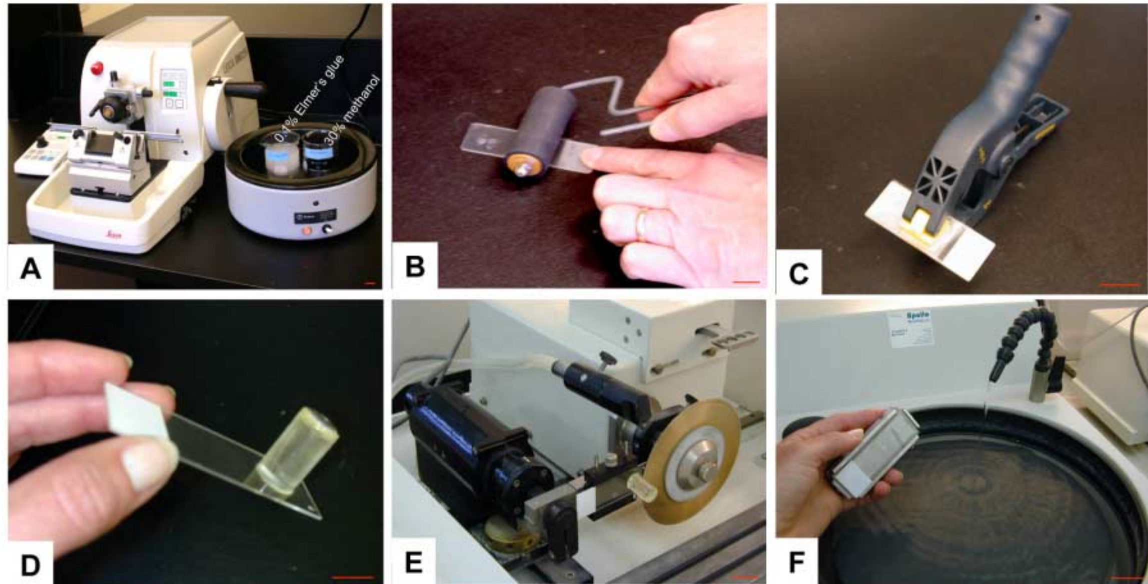

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Figure 2

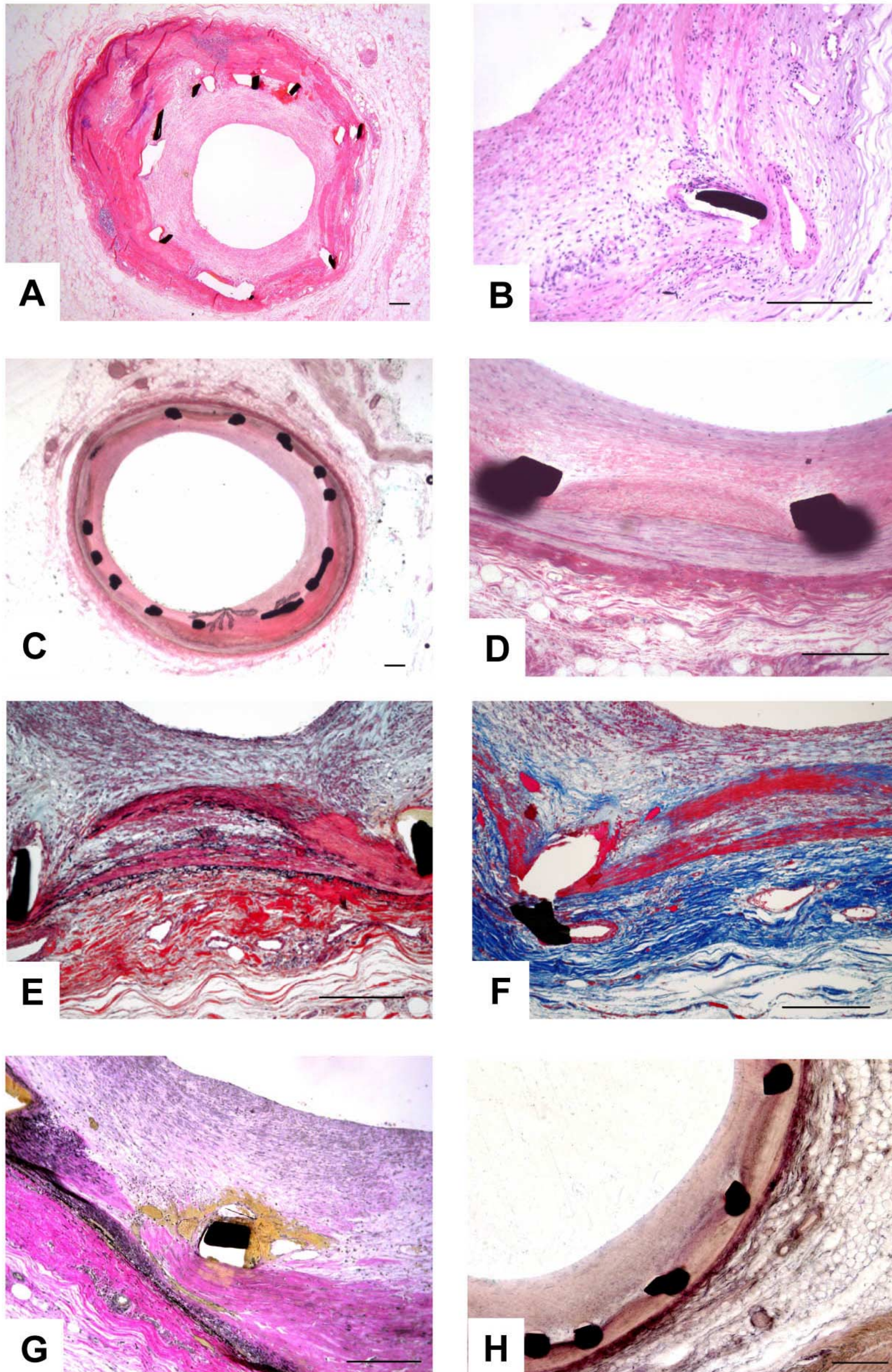

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Figure 3

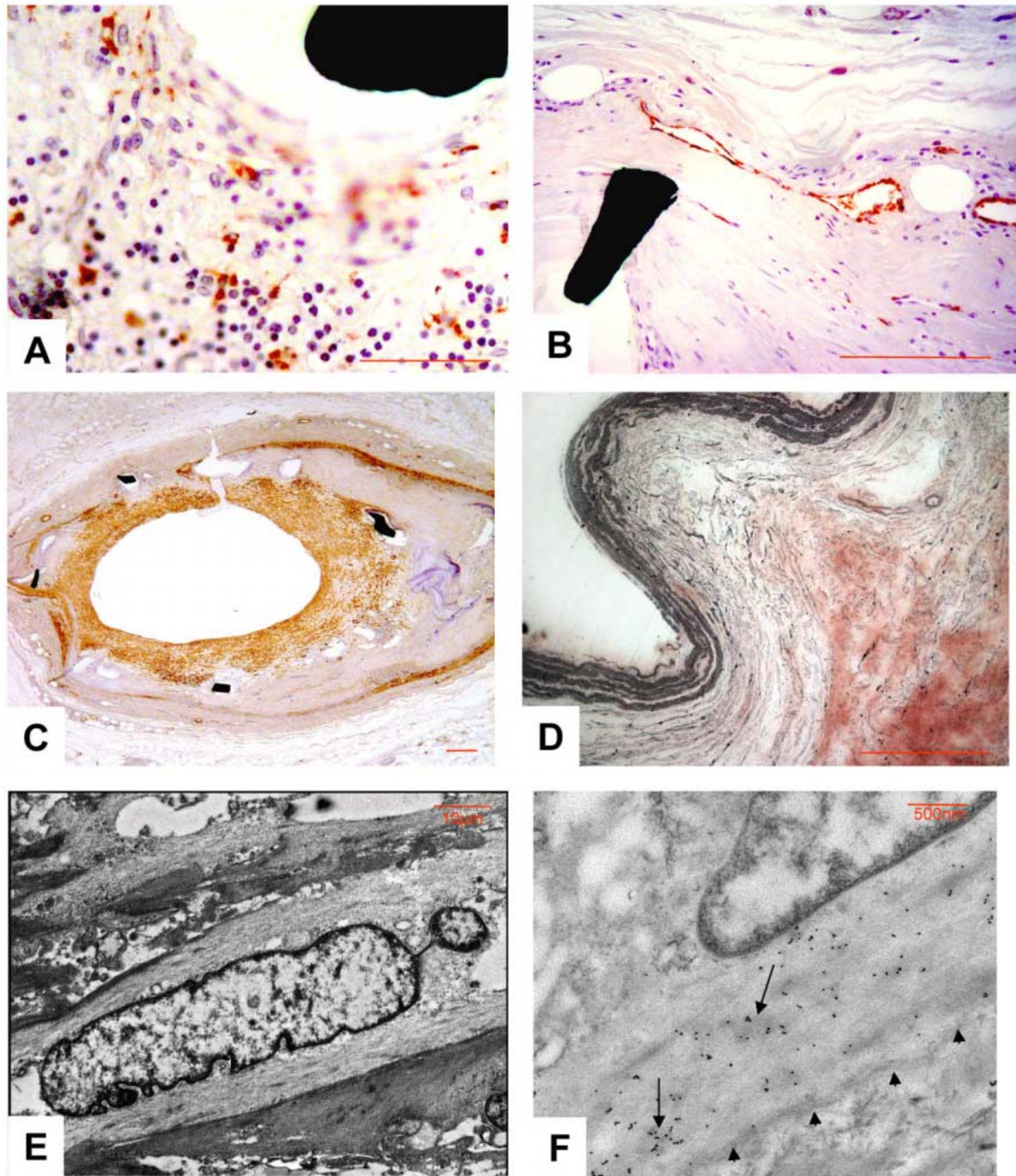

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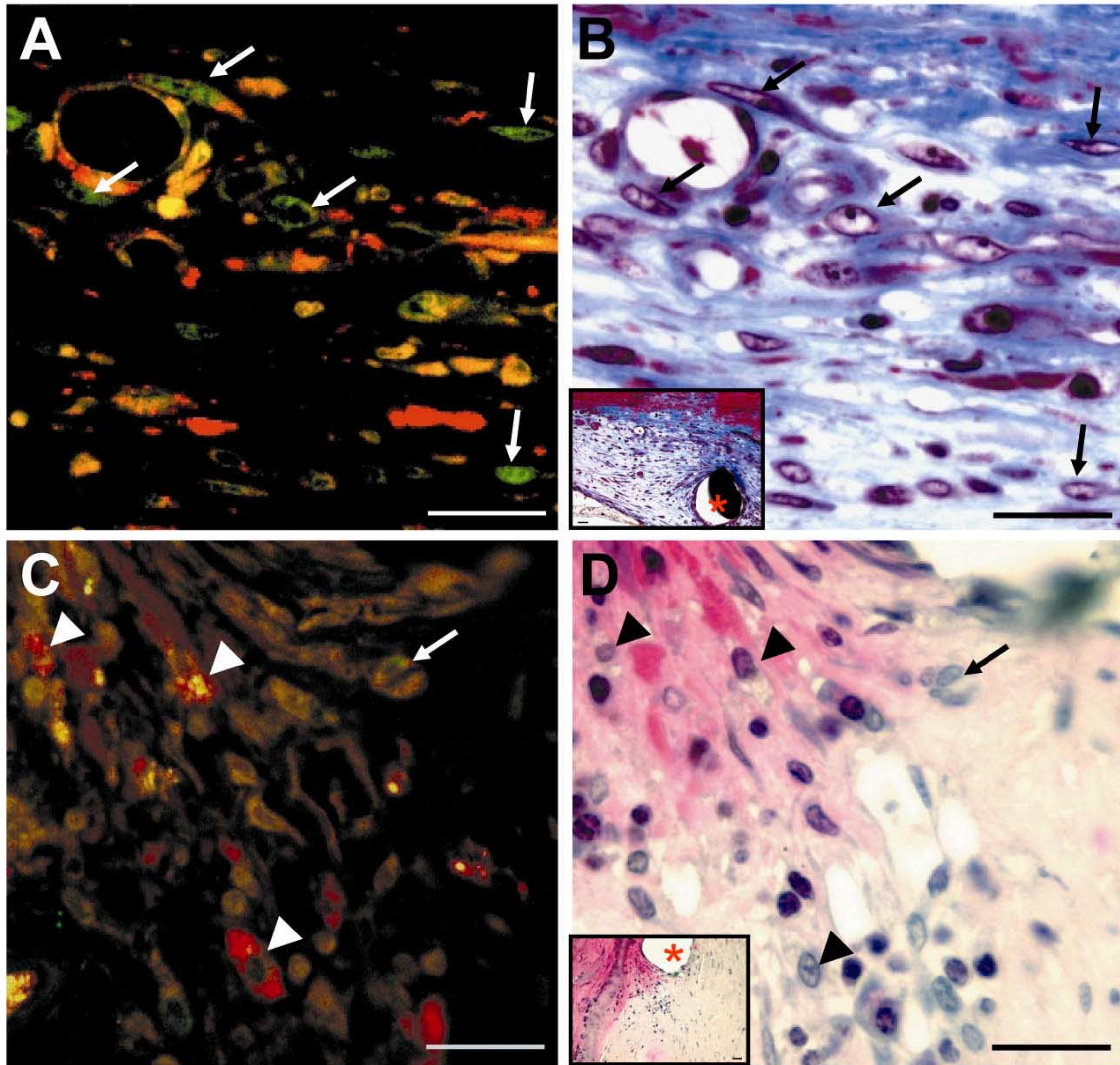
Figure 4



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Supplemental Material

Supplemental Table 1: Processing Schedule for Stented Tissue Embedded in MMA

Step No.	Solution	Concentration	Time (minutes)	Temp (ºC)
1	Ethanol	80%	30	4
2	Ethanol	80%	30	4
3	Ethanol	90%	30	4
4	Ethanol	90%	30	4
5	2-propanol	100%	60	4
6	2-propanol	100%	60	4
7	Toluene	100%	60	4
8	Toluene	100%	60	4
9	Toluene/Resin Sol I	50%	60	4
10	Resin-Sol I	Pure	Overnight	4
11	Resin- Sol I	Pure	All day	4
12	Resin- Sol II	Pure	Overnight	4
13	Resin- Sol II	Pure	All day	4
14	Resin- Sol III	Pure	Overnight	4
15	Resin- Sol III	Pure	All day	4
16	Polymerization mixture			-18 to -20

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Supplemental Table 2: Current Equipment Cost Estimation

TC Method	SG Method
Leica RM 2255 rotary microtome: $14,267.10 (U.S.) D profile tungsten carbide knife: $ 1,226.11 (U.S.) [a]	Beuhler MetaServ 2000 Grinder/ Polisher: $6,388.44 (U.S.) Beuhler IsoMet 5000 Linear Precision Saw: $26,201.20 (U.S.) Wafering Blade & ancilliary supplies: $2,499.00 (U.S.)
Total: $15,493.21 (U.S.)	Total: $35,089.32 (U.S.)

[a] It must be noted that knife damage is inevitable when sectioning stented arteries therefore extra knives and resharpening fees should be taken into account.

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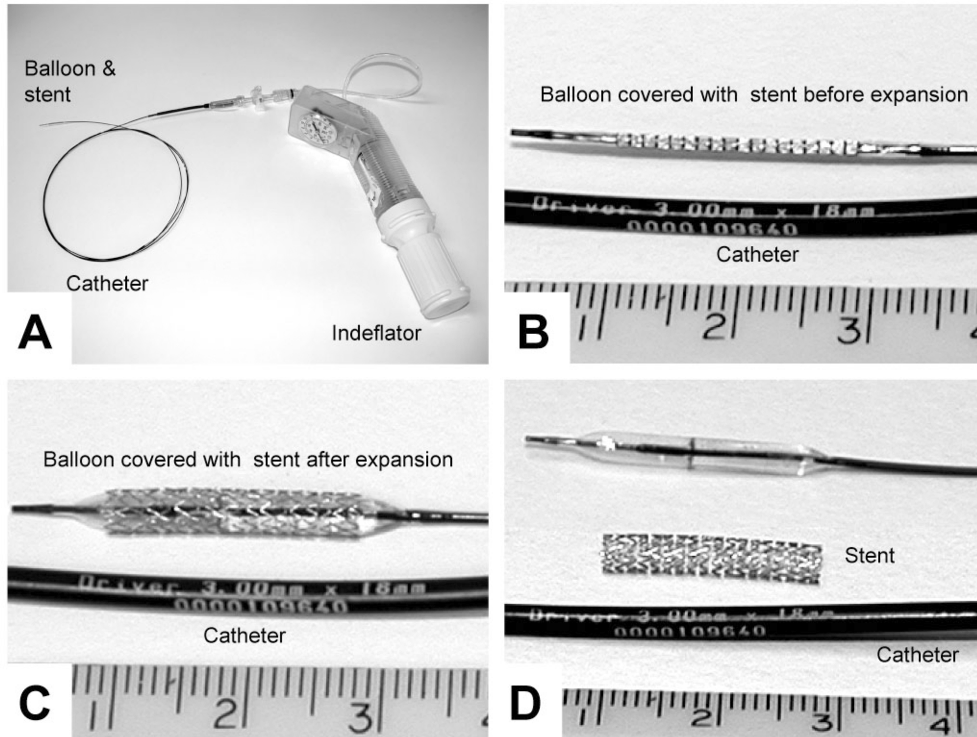
Supplemental Figure 1



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