

SUM NOTES

Preparation of Bone Cross-Section with Implants

Introduction

In the last decade, considerable technological improvements have been made to repair damaged bones and tissue. Devices such as hip implants and stents are being used extensively to repair and correct problems, which were difficult to repair in the past.

Biocompatibility of the implant in the body is a major concern and the main reason for microscopic examination. It is necessary for a successful implantation that the bone or the tissue accepts the implant and does not reject the intrusion. While at the same time, surgical implants are exposed to the biochemical environment of the body, which can cause degradation. In order to evaluate the compatibility, it is important that any preparation technique for microscopic analysis prevent damage to the bone or the tissue and at the same time maintain the integrity of the implant.

Bone is spongy, can regenerate and thus can change volume with time. Implants on the other hand are hard, do not change volume and can degrade in the body causing failure. The dissimilarity of the material hardness, i.e. a hard implant versus soft bone or tissue, makes specimen preparation a challenge.

Bones and tissues are studied by two different methods. Thin sections are used for microradiography and for observation with transmitted light. Cross-sections of bulk specimens are observed with reflected light. Thin sections are much more common and provide considerably more information than bulk specimens and will be discussed in detail.

Thin Sections

A polished thin section containing an implant may be used for both transmitted and reflected light examination. The implant, which is opaque, is examined by reflected light for features such as porosity, grain size and corrosion. The bone or tissue, which are transparent, are examined in transmitted light. Cellular features, such as osteons and haversian canals, show their structural details more clearly in a thin section. Features, such as pores, coatings (HA) and corrosion in metallic implants are better observed with a polished section.

Generally, the paraffin method is used to prepare decalcified bones and soft tissues where the specimen is sliced with a microtome. However, a microtome cannot be effectively used for bone or tissue that contains an implant, because the microtome knife can be easily damaged. A damaged knife can cause tearing of tissues, damaging the specimen. When sectioning decalcified bone, the microtome may also cause folds, shrinkage or micro cracks, making microscopic examination difficult.

Preparation Procedure

1. Cut the specimen to create an approximately 2mm thin section,



Microstructure of a bone thin section in transmitted light. Dark area is the implant.

preferably using a precision diamond saw. Wash, thoroughly dry, and embed the specimen in EpoThin® Low Viscosity Epoxy Resin under vacuum.

2. When cured, grind one side with 600 and 800 grit (P1200 and P1500) CarbiMet® Abrasive Discs until the entire surface is exposed and flat.
3. Clean and dry the specimen and a slide thoroughly. Attach the ground side to the slide using epoxy or cyanoacrylate glue. Cyanoacrylate must be used very carefully because it cures very rapidly and can bond fingers to the slide if not handled properly. Cyanoacrylate glue works best when used with plastic slides. When using a glass slide, grinding the surface with 600 grit (P1200) abrasive before attaching the specimen, will improve the adhesion.
4. When using epoxy to obtain a good and uniform thin bond, place the slide in the PetroBond™ to apply pressure while curing, or apply pressure by other means. Make sure that the epoxy does not come in contact with any part of the bonding jig. Place the PetroBond™ on a hot plate at 113-122 °F (45-50 °C) for 2-3 hours. Heat will accelerate the cure cycle. If heat is not applied, the epoxy may take 24 hours or longer to cure.
5. When the epoxy has cured completely and cannot be indented by a fingernail, remove and cool the slide to room temperature.

- For medical applications, specimens containing implants should be cut first with an IsoMet® Precision Saw, then ground with the PetroThin® Thin Sectioning System. Cutting the specimen with one of the IsoMet® Saws will not only conserve the specimen but minimize tissue damage. The thicker blade required for PetroThin® may cause damage to the implant and the tissue.

If the specimen has to be ground by hand use coarser abrasives such as 320-400 grit (P400-P800) CarbiMet® Discs. Grind the specimen to approximate thickness of 125µm. Reduce the thickness further by grinding with finer abrasive such as 600 and 800 grit (P1200-P1500) CarbiMet® Discs until approximately 20-50µm in thickness or until the cellular details become clear. One has to be extremely careful when grinding a specimen less than 50µm in thickness. Note: *For micro-radiography a specimen approximately 80-100µm thick is suitable; but for observing with transmitted light a thin section should be between 20 and 50µm in thickness, or ground until the cellular details become clear for observation.*

- Polish on TexMet® 1500 or Nylon Polishing Cloth using 3µm MetaDi® Diamond Paste. Use MetaDi® Fluid as the lubricant.
- If required the specimen can be final polished on MasterTex® Polishing Cloth with MasterPrep™ Alimina Polishing Suspension for a short time to remove the scratches.

When preparing a thin section it is useful to maintain the top and the bottom surface parallel to each other, especially if the specimen is going to be used for micro-radiography or for examining with a transmitted light microscope. To maintain parallelism, prepare the specimens with semi-automatic machines such as the PetroThin® Thin Sectioning System, or by hand on a rotating wheel using special holders. The PetroThin® Thin Sectioning System is designed to prepare thin sections of precise thicknesses. Its use assures consistent results.

Equipment*

IsoMet® Family of Linear Precision Saws

PetroBond® Thin Section Bonding Fixture

PetroThin® Thin Sectioning System

Consumables*

IsoMet® Diamond Wafering Blade

EpoThin® Low Viscosity Epoxy

CarbiMet® Abrasive Discs

TexMet® 1500 Polishing Pad

MetaDi® Diamond Paste (3µm)

MetaDi® Fluid

MasterPrep™ Alumina Polishing Suspension

*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

To minimize some of the problems mentioned above with manual preparation, use of a holder such as the Histologic Precision Grinding Fixture (part number 60-8087) helps. The fixture has carbide stops, which prevent the specimen from being over ground. The fixture is designed for slides that are 27 x 46mm in size and cannot accommodate slides of other sizes.

If the specimen is prepared by hand without the aid of a special holder, the following problems are encountered.

- Lengthy preparation time
- Non-uniform results
- Difficulty in maintaining uniform thickness

Sectioning AbrasiMet • AbrasiMatic • IsoMet	Mounting SimpliMet	Grinding & Polishing EcoMet • AutoMet • MetaServ	Imaging & Analysis OmniMet	Hardness Testing Wilson® Hardness
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