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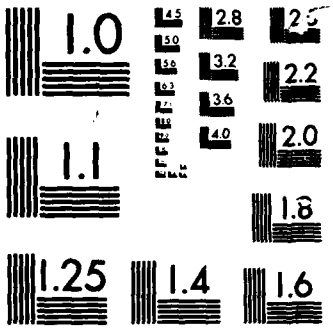

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A THICK-SECTIONING TECHNIQUE FOR PRESERVATION OF BONE-METAL INTERFACES

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A technique is described for preparing 100-150 micrometer thick-sections of bone plus metallic implant that has been embedded in polymethyl methacrylate (PPM). Sectioning is accomplished by using a low speed saw. PMM is removed using a xylene and eosin wash (80:20 V:V) and sections are fixed on glass slides using Eukitt's mounting medium. Bone-metallic specimens prepared in this manner allow for optimal visualization of the bone implant interface. Moreover, microscopic assessment of attendent soft and hard tissues for histomorphometry is facilitated.

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ABSTRACT

A technique is described for preparing 100-150 micrometer thick-sections of bone plus metallic implant that has been embedded in polymethyl methacrylate (PPM). Sectioning is accomplished by using a low speed saw. PMM is removed using a xylene and eosin wash (80:20 V:V) and sections are fixed on glass slides using Eukitt's mounting medium. Bone-metallic specimens prepared in this manner allow for optimal visualization of the bone implant interface. Moreover, microscopic assessment of attendent soft and hard tissues for histomorphometry is facilitated.

Key Words: Implants, Bone

INTRODUCTION

Stainless steel and titanium alloys are being used with increasing frequency for maxillofacial reconstructive surgery. The presence of metal in osseous tissues complicates the preparation of microscopic specimens for routine histologic examination and for histomorphometry. The clinical success of the metallic implant requires an obligatory contiguous osseous interface. Developmental research leading to new and better metallic bone implants is requisite upon an unencumbered and crisply resolved osseous-to-metal boundary. Conventional microtomy techniques and manual grinding are unsatisfactory because the critical assessment zones are destroyed. Furthermore, hand grinding leaves metallic filings that often obliterate essential histologic and histomorphometric features.

This paper describes a method that uses a Buehler Isomet Low Speed Saw for producing 100-150 micrometer thick sections of PMM-embedded, undecalcified bone-metal sections. The removal of the PMM with a xylene-eosin wash allows for enhanced visualization of important histologic features.

MATERIALS AND METHODS

Following removal, a bone-implant specimen is immediately placed into 70% ethanol and cut into 0.5-1.0 cm sections using a Buehler Isomet Low Speed Saw. Specimens are kept in a standard temperature refrigerator until embedding. The specimen is taken through a series of ascending ethanol washes followed by infiltration and embedding in PMM.

Specimens are sectioned at 100-150 micrometers using the Buehler Isomet Low Speed Saw and a diamond wafering blade. Sections are placed on a clean glass slide with 70% ethanol and covered with a strip of polyethylene. The slide is then warmed on a warming tablet (50 degrees Centigrade) for 12 hours. A lead weight applied to the slide insures a wrinkle-free specimen. The slide is subsequently examined for suitability of histologic preparation. The specimen is carefully removed with a number 10 scalpel blade and permanently mounted to a clean glass slide with 2-3 drops of Eukitt's mounting medium and allowed to set 12 hours (see Fig. 1.). A xylene-eosin wash (80:20 V:V) is carefully used to completely cover the slide. The specimen is deplasticized for 24-48 hours, then coverslipped in the usual manner with Eukitt's mounting medium. A coverslip and lead weight are applied for 24 hours.

RESULTS

The protocol described produced bone-metal sections that are highly compatible with routine histologic examinations and for histomorphometry. Extremely important interfacial boundaries were preserved (see Fig. 2). Additionally, fibrous tissue invasion of the interface was easily characterized with Eosin. Microscopic evaluations of osseointegrated dental implants may be greatly facilitated with this technique.

Fig. 1. Thick-section following removal of the plastic.

Fig. 2. Bone-implant junction (40X) showing bone growth and eosinophilic fibrous tissue adjacent to the metal implant.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

Commercial materials and equipment are identified in this report to specify the investigative procedure. Such identification does not imply recommendation or endorsement, or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the U.S. Army Medical Department.

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