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A Method for Cemented Bone Interface Examination Without Polymethylmethacrylate Embedment

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Abstract

Although effective, the embedment of bone tissue and orthopaedic devices using polymethylmethacrylate (PMMA) has challenges and limitations. To embed using PMMA, specimens must first be fixed in 70% ethanol, dehydrated in ascending grades of ethanol, and then infiltrated and polymerized in polymethylmethacrylate using standard techniques. This process can take more than 22 d for large bone specimens. Additionally, PMMA embedment has been shown to dissolve bone cement, thus enabling the analysis of the bone–cement interfaces. To conserve processing time while preserving the bone–cement interface, a method was developed for processing mineralized bone tissue in preparation for scanning electron microscopy (SEM) imaging that does not require PMMA embedment. This technique does not require the traditional dehydration and PMMA polymerization process. Instead, fresh mineralized cemented bone specimens were serially sectioned and the marrow removed after formalin fixation. The sections were air-dried then desiccated. The sections were then prepared for SEM imaging and examination. This process takes a fraction of the tissue processing time while not compromising the bone–cement integrity. The SEM image quality was shown to be comparative to images obtained with PMMA-embedded bone specimens.

Keywords

bone; embedding; implants; polymethylmethacrylate; orthopaedics

Introduction

In the past, orthopaedic research efforts have focused on quantifying the bone cement area and cement penetration into bone following total joint replacements. This information contributes to a better understanding of cement–bone interactions in achieving durable skeletal attachment. It would be helpful if quantitative analysis could be done on mineralized cemented bone specimens using scanning electron microscopy (SEM) imaging without embedding the specimens. Before SEM imaging, it is common practice to embed mineralized bone with implant in situ in polymethylmethacrylate (PMMA) (1–5).

Because embedment using PMMA is the traditional method for preparing mineralized bone tissue and implant for interface analysis after microscopy, the protocol is well established (6–8). The PMMA-embedment procedure enables fixation of mineralized bone specimens with orthopaedic implants in situ. PMMA specimens can be further ground for light microscopic analysis. However, the PMMA process can take more than 22 d (9) and

dissolves the bone cement, seriously compromising quantitative bone–cement interface analyses. A simplified method for processing mineralized bones with cemented implants in preparation for SEM analysis could prove to be beneficial in assessing the durability of cemented fixation in bone. Thus, the objective of this study was to examine a simplified process for preparing mineralized cemented bone-implant specimens without the use of PMMA while not compromising the bone–cement interface or the quality of images captured during scanning electron microscopy.

Methods

Institutional review board approval was obtained before the start of this investigation to allow for implant retrieval at the time of autopsy. Two different specimen types were tested in this study. The first specimen consisted of one human proximal tibia that was retrieved from a postmortem donor. This donor had a cemented cobalt chrome alloy component. The second specimen type consisted of seven cadaveric tibias cemented to titanium alloy implants. All of the specimens consisted of human proximal tibias measuring approximately 6 inches in length. The specimens were secured in a vice and sectioned on a high-speed water-cooled saw equipped with a diamond blade (Rockazona, Peoria, AZ) into 2- to 3-mm thick sections (2,6,7,9). Eight to ten sections were made along the coronal plane of the titanium alloy specimens and eight sections made from the cobalt specimen (Figure 1). After sectioning, the bone marrow was removed from the sections using a lavage high flow water tip and suction tube (Stryker Instruments, Kalamazoo, MI). Air drying was accelerated using a standard household hair-dryer then further dehydrated overnight in a desiccator containing Dry-Rite™. Once dry, the sections were sputter-coated with a conductive layer of gold for three minutes (Hummer VI-A; Anatech, Alexandria, VA). Sections were placed individually into the scanning electron microscope (JSM 6100; JEOL Inc, Peabody, MA) equipped with a backscattered electron detector (Tetra, Oxford Instruments Ltd, Buckinghamshire, UK) and associated image capture software (ISIS 300 series, Oxford Instruments Ltd.). Images were captured between 20 and 400 times magnification that were of research quality to enable quantitative analysis.

Results

The new technique shortened the time necessary to process cemented implants in-situ from more than 22 d to less than 24 h. The quality of the SEM images were not compromised (Figure 2). Quantitative measurements could be made using the same imaging technique previously used for bone ingrowth analysis (2) from PMMA embedded specimen images. The bone marrow was removed, and thus complications from specimen charging in the SEM was avoided. The bone–cement implant interface was preserved.

Discussion

After a careful literature search, it appeared that there are limited microscopic quantitative studies (10) on the bone–cement surface, host bone contact, and bone penetration into the bone cement in total joint replacements. Understanding skeletal attachment with bone cement has been shown to be important for understanding the reasons for cement fixation success or failure. The bone cement appeared to work similarly to an embedment media to protect the bone cement interface. Examination of the surrounding bone appeared to be structurally intact but studies such as microfracture analysis would obviously be negated by this processing technique. Subsequent cellular studies for bone cell response and foreign body reactions or other cellular assessments would be compromised and could not be performed after using this technique. Hypothetically, fluorochrome analysis may be possible with subsequent PMMA embedment, but future studies are required.

Conclusion

The development of this technique can provide additional information on the bone–cement interface skeletal attachment process in total joint replacement. Investigators need to keep in mind the compromises that occur when using this technique.

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Figure 1.
Microradiograph of 2- to 3-mm thick tibial section showing preservation of the bone–
cement interface after sectioning.

Figure 2.
SEM image of the bone–cement interface illustrating the ability to achieve quality SEM images of bone and cement for use in quantitative analysis without PMMA embedment.