H146 A Comparison of Three Methods for Assessment of Bone Decalcification Time and Quality of Histological Slides for Cranial Fracture Healing Investigation

Jered B. Cornelison, PhD, Western Michigan University School of Medicine, Kalamazoo, MI 49008; Carolyn V. Isaac, PhD, Kalamazoo, MI 49008-8074; Joseph A. Prahlow, MD, Western Michigan University School of Medicine, Kalamazoo, MI 49007*

Learning Overview: After attending this presentation, attendees will: (1) learn an easy, accurate, and non-destructive method for determining whether bone samples are decalcified; (2) learn the amount of time required to decalcify cranial vault bone using three different decalcification methods among three different age cohorts; and (3) understand which decalcification methods affect the quality of bone histomorphology.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing best practices for human bone decalcification aimed at investigating the microscopy of bone and fracture healing. As such, the results of this study will be used as a standard protocol for decalcification of cranial bone from various aged decedents.

As part of a National Institute of Justice grant investigating cranial fracture healing, several decalcification techniques were explored to determine the amount of time for processing and the resulting quality of the healing bone histomorphology. Forensic histology laboratories often use Hydorochloric acid (HCL) because it provides the most expedient bone decalcification. However, there is evidence, that nitric acid and Ethylenediaminetetraacetic acid (EDTA) decalcification yield better results in non-human bone for the visibility of tissues, cells and molecules active in bone biology.[1] As most studies of cranial bone decalcification and healing use small animal models, this is an important first step to determine the methods best suited for human bone decalcification.

The sample is comprised of 50 samples from 23 individuals, ranging in age from 2 months to 77 years. Samples were fixed in 10% formalin for two to four weeks. Before decalcification, all bone sample dimensions and weights were recorded. Where possible, bone samples were cut into three sections for immersion in 10% EDTA, 5% nitric acid, and 7% HCL, and placed on an orbital shaker. Radiographic techniques were used to determine the degree of decalcification. In the initial radiographic inspection, settings were adjusted until radiopacity was consistent across the bone sample. These same settings were used in subsequent examinations until radiopacity was absent throughout the sample, indicating decalcification was complete. It was found that the radiographic technique varied primarily according to bone thickness and, secondarily to age. Thinner samples, such as infant cranial bone and thin areas of adult bone (e.g., pterion), required a setting of 40KVp to 48KVp and 0.9mAs to 1.8mAs. Older children and diploic bone required a setting of 50KVp and 2.5mAs.

After decalcification was complete, samples were rinsed in water for at least an hour to halt the decalcification process. Each sample was then cut with a scalpel and placed in a cassette for paraffin embedding and slide preparation. Three different histological stains were used, including Hemotoxylin and Eosin, Masson's Trichrome, and Alcian Blue Hematoxylin with an Orange G counterstain, resulting in 150 histological slides. Slides were assessed for the quality of cell, cell nuclei, and tissue visability.

The results of the decalcification study show that for infants, juveniles, and adults HCL decalcification was the most rapid technique, followed by nitric acid and EDTA. For infants, HCL required 1 to 2 days, Nitric Acid required 2 to 5 days, and EDTA required 5 to 27 days for decalcification. For juveniles, HCL required 4 days, and nitric acid required 7 to 8 days for decalcification. Adult samples were more variable, requiring 2 to 4 days with HCL, 3 to 10 days with nitric acid, and 13 to 60 days with EDTA. EDTA decalcification times tended to increase with age; older aged adults required more decalcification days. A review of slide quality indicates that nitric acid and EDTA yield optimum results, with highly visible nuclei and greater stain color gradient contrast than HCL.

In conclusion, for high-quality slides produced relatively rapidly (1 to 10 days), nitric acid is the best decalcification technique for viewing histological structures related to bone. However, the gentle effects of EDTA chelation allowed samples to be used for immunostains and other special stains useful for investigating cells and tissues involved in bone healing.

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Reference(s):

1. González S.A., Pacheco-Tena C., Macías-Vázquez C., Luévano-Flores E. Assessment of Different Decalcifying Protocols on Osteopontin and Osteocalcin Immunostaining in Whole Bone Specimens of Arthritis Rat Model by Confocal Immunofluorescence. *Int. J. Clin. Pathol.* 2013 (10): 6 1972-1983.

Decalcification Methods, Cranial Bone Histomorphology, Cranial Fracture Healing