Abstract 3: Society of Biomaterials; 1990.

RELATIONSHIP OF GRAYLEVELS IN BACKSCATTERED ELECTRON IMAGES OF SIMULATED BONE TISSUE TO ATOMIC NUMBER, DENSITY, AND MINERAL CONTENT

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INTRODUCTION: For several decades the backscattered electron (BSE) mode of the scanning electron microscope (SEM) has been used to obtain information about composition and crystal structure of pure and composite materials. Recently, this technology has been used to study bone mineralization. Specifically, BSE images have been analyzed to give an indication of relative differences in mineral content of bone tissue [1].

Observations using the BSE mode show that bone exhibits different morphologies and various degrees of mineralization or mineral content. Mineral heterogeneity is observed in BSE images as changes in graylevel. Darker areas represent newly formed tissue, which is presumably of lower mineral content and areas of whiter graylevel where tissue is mature are presumably of higher mineral content. However, the relationship between mineral content and graylevel changes has not been rigorously demonstrated. Many authors have described BSE image graylevels as being a function of mineral density, mineral content, density [1], or mass density [2], although these terms cannot be used interchangeably. This ambiguity has lead to confusion when interpreting the role of BSE images in bone mineral analysis.

The main objective of ths investigation is to demonstrate the relationship of average graylevel to average atomic number (Z), density (g/cc), and mineral content (volume fraction). To accomplish this objective, simulated "bone tissues" were prepared by mixing minerals of known composition and crystallinity with a collagen simulating epoxy glue.

MATERIALS AND METHODS: Composite mineral mixtures were made by combining bone substitute minerals with epoxy glue. Hydroxyapatite (HA = 3.14 g/cc), octacalcium phosphate (OCP = 2.8 g/cc), and brushite (B = 2.4 g/cc) in 30%, 40% and 50% proportions by volume were used. The average atomic number of each mineral was calculated. The average atomic number of epoxy was considered to be equivalent to that of Type I collagen.

Volume percent mixtures were prepared by combining by weight the appropriate amount of epoxy and mineral in a clean plastic weighing boat, and manually mixing with a small spatula for 20 to 30 minutes. The resulting homogeneous paste was compressed into holes previously drilled into a plastic block. After curing, the plastic block containing the mixtures was ground and polished to an optically smooth finish.

The plastic block was lightly coated with gold, mounted flat on a metal base, and placed in the chamber of the SEM at 0° tilt. To insure uniform operating conditions, calibration procedures were performed at the beginning of the imaging session and every 20 minutes using a magnesium standard [3].

Two randomly selected fields of each mixture were captured at 200x and stored using an image analysis system. Graylevel analysis of each material was conducted on stored images after all images had been captured. Each image was systematically analyzed in ten separate segments representing a total of 70 percent of the image area. The graylevels were represented using backscattered electron profile (BSEP), which is an outline of a histogram distribution of graylevels in selected areas [3]. Data from the ten BSEPs of each material mixture were then combined into one representative or "aggregate" BSEP. An average graylevel was determined [3].

RESULTS AND DISCUSSION: Figures 1 and 2 illustrate how BSE graylevels can change markedly by altering only mineral content. Changes in mineral content produce changes in both density and average atomic number of the mixture (Fig. 1).

Figure 2 shows aggregate BSEPs of mixtures of varying mineral content. The two 40% mixtures (10 B/30 HA and 40 HA) and two 50% mixtures (HA and OCP) demonstrate how image graylevels can change by altering composition, even when the same volume fraction is maintained. Using equivalent volume percentages of different materials has the effect of changing the average atomic number (Z) of the mixture, resulting in higher or lower graylevel in the BSEP.

The results of this study demonstrate that in simulated bone tissue, the main factors influencing the changes in graylevel and resulting BSEPs is the volume fraction of mineral. This is referred to as mineral content. Previous suggestions that changes in graylevel are solely related to mineral density (g/cc) [1] were not supported by this study. Another influence observed to affect the graylevel signal was the compositional changes. The addition of lower atomic number OCP and brushite effectively lowered the graylevel of mixtures with equivalent volume fractions. Therefore, future interpretations of BSE graylevel changes in bone should not be directly attributed to mineral density, density, or mass density because of the ambiguity inherent in these terms. The proper interpretation of changes in bone graylevels may best be attributed to mineral content changes (volume fraction) since there is a direct correlation with mineral content and the average atomic number of the bone substitute materials used in this study (Figure 3).

The application of BSE image graylevel analysis should continue to prove useful in studies assessing mineral content at a microscopic level in normal maturation and aging bone, and in assessing the response of bone to biomaterials.

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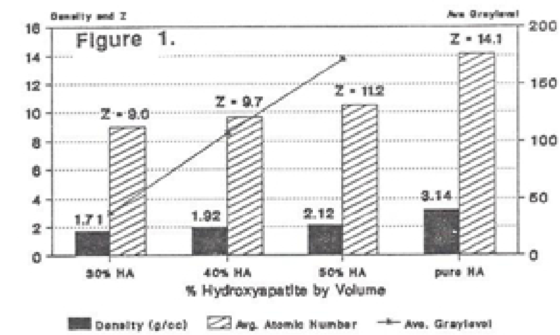

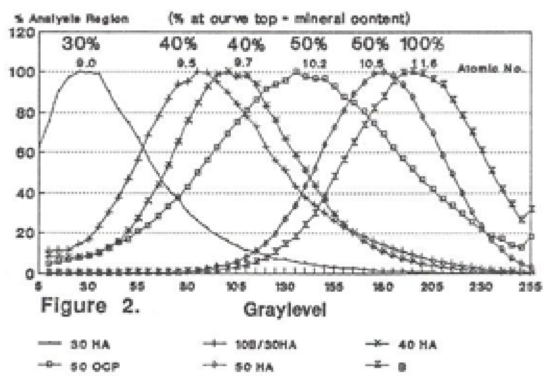

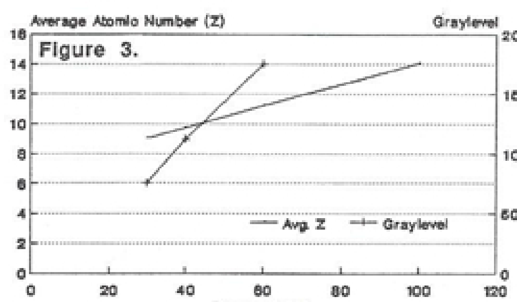

ACKNOWLEDGMENTS: We would like to acknowledge the support of the Department of Veterans Affairs Medical Center, Salt Lake City, UT, and the Whitaker Foundation.