An Assessment of the Prevalence of Organic Material on Bone Surfaces

Jade Chow and Timothy J. Chambers

Department of Histopathology, St. George’s Hospital Medical School, Cranmer Terrace, London SW17 ORE, U.K.

Received January 9, 1991, and in revised form March 14, 1991

Summary. Although an unmineralized layer of organic material has been identified on both bone-forming surfaces and surfaces upon which bone formation has ceased (quiescent surfaces), the proportion of bone surfaces that is covered by unmineralized material has not been quantified. Because the unmineralized layer may play a role in the regulation of bone resorption, we undertook a scanning electron microscopy (SEM) assessment to determine its extent. Specimens of adult human ribs were prepared for undecalcified resin sections and SEM. For SEM, cells were removed and the bone surface was inspected and photographed. The same specimen was then immersed in NaOCl to remove organic material, and inspected again in the SEM. We found that the surface of bone appeared quite different before, compared to after, removal of organic material. Before removal, the entire nonresorptive surface was finely fibrillar. After removal of the organic material we observed a minor component showing the finely nodular surface typical of mineralizing bone, and a major component in which the mineral surface was free of such nodules. In only 3 of 1,200 photographs did we identify areas in which the bone surface was not altered by removal of organic material from the specimen. Analysis of histological sections of the ribs showed that approximately 85% of the bone surface was classifiable by light microscopy as quiescent. These results suggest that not only formative but also quiescent surfaces are covered by a layer of unmineralized organic material.

Materials and Methods

Human 6th ribs were obtained from individuals (ages 28–92 years, mean 62.5 years) at postmortem within 24 hours of death. Their clinical histories and autopsy findings did not suggest any significant bone disease. Each rib was bisected into medial and lateral halves, and each half into several pieces.

Pieces of rib (~ 5 x 5 x 2 mm) were processed to remove organic material from the surface (‘anorganic specimens’). Organic material was removed with 10% NaOCl (4 hours), washed with phosphate buffered saline (PBS) (pH 7.4), and then fixed in 3% glutaraldehyde buffered to pH 7.4. The specimen was dehydrated through alcohols, air-dried, and then sputter coated with gold for SEM using a Cambridge S90 SEM. Morphometry of this specimen was performed by counting quiescent, formative, and eroded surfaces using a perspex...
Fig. 1. Scanning electron micrograph of human rib showing a typical quiescent surface on the left of the photomicrograph. The surface consists of fine fibrils, which are generally parallel, but in places show an intersecting pattern. To the right of the quiescent surface is a resorption surface comprising numerous coalescent excavations with sharp elevated margins. In the floor of the excavations are parallel bundles of partially demineralized fibers. Magnification ×320.

Fig. 2. Approximately 10% of the surface of the ribs was revealed, by treatment with NaOCl to remove organic material, to be formative. Formative surfaces were distinguished by the presence of tiny amorphous nodules, often 1 μm in diameter or smaller, on the surface of the specimen. Magnification ×320.

sheet with four equidistant points over the monitor screen, scanning at a screen magnification of 1,000×. Three thousand (covering at least 15 mm²) were counted for a specimen from each of the 7 individuals. The criteria used to identify these surfaces are given in the results section.

'Organic' specimens (i.e., specimens prepared without prior removal of the organic component) of the same rib were also prepared in the following manner. The marrow was washed away with a water jet until the specimen appeared clean. The surface cells were removed by immersing the specimen in 0.25 M NH₄OH (15–20 minutes), followed by washing in PBS. The ribs were then fixed in buffered 3% glutaraldehyde and prepared for SEM as above, except