CHARACTERIZATION OF MICROINDENTATION IN BONE VIA SCANNING ELECTRON MICROSCOPY

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INTRODUCTION

The objective of this work was to characterize cross sectional profiles of microindentations in wet and dry bone specimens to better understand material behavior at the indentation site. Micro and nano indentations in ceramics, metals, and polymers have been characterized and an understanding of the underlying mechanical processes developed. A number of researchers include a description of the indentation cross section [4,5]. Typically, a sketch is provided in the paper describing the cross section shape at full load and the residual impression at complete unload (**Figure 1**). The residual impression is shown with an apex angle larger than that of indenter tip. The indenter is in contact with the specimen until the very tip leaves the specimen. The difference in depth between the full load and full unload is related to the elastic properties of the material. This method was pioneered by Loubet [3] and used on bone by researchers following an improved method [4]. The residual impression walls are shown as straight and a zone of material pile up indicated at the edge of the impression.

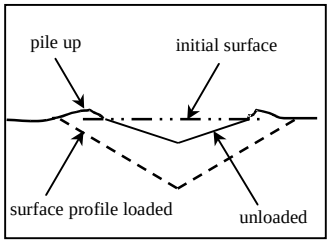

Figure 1. Typical schematic of theoretical treatments.

The question of whether or not this idealized model represented the situation in bone arose. Additionally, the microindentation cross section of wet and dry bone specimens has not been previously characterized as far as we are able to determine. Such characterization can help elucidate the mechanical behavior of the bone tissue. Moreover, it can lead to a deeper understanding of exactly what is being measured during microindentation and whether or not the assumptions made in theoretical treatments are entirely correct for bone.

METHODS

One bovine right metacarpus was longitudinally sectioned from the distal dorsal aspect to produce a one millimeter thick by 25 mm wide by 45 mm long slab. All procedures were approved by our Institutional Animal Care and Use Committee. The slab was further sectioned into two microindentation specimens. Each specimen was polished in a progressive manner with a 6 μm diamond slurry, a 3 μm diamond slurry, and final polish with 0.05 μm alumina and colloidal silica suspension. The polished specimens were indented in arrays on a set pitch (**Figure 2**) using a Knoop pyramid. The first specimen was indented dry with a test mass of 200 g and a dwell time of 10 s. The second specimen was indented wet with a test mass of 100 g and the same dwell time. All specimens were first imaged in an optical microscope and then sputter coated for imaging in the SEM using 15 kV excitation.

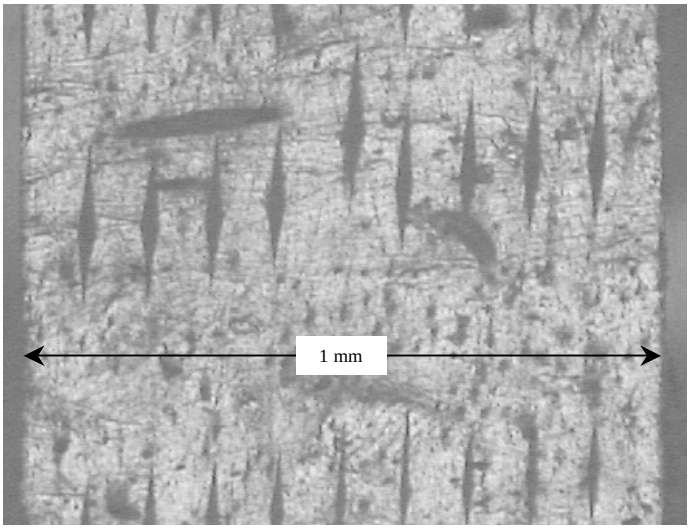

Figure 2. Typical set of microindentations.

RESULTS

The first of four notable findings was that the residual impression wall angles, as measured on all four specimens, were greater than that imposed by the indenter tip geometry of 130° (**Figure 3**). Second, no pile up existed on the short diagonal sides of the residual impression where the walls meet the initial material surface (**Figure 3**). Third, the wall angles were larger for the wet specimens than for dry. Fourth, cracks existed along the apex of long axis of the microindentation in both cases of wet and dry specimens (**Figure 4**). Also notable was the unevenness of the polished specimen surface and lack of well defined edge or boundary between the impression and the initial surface (**Figure 4**).

DISCUSSION

The residual impression wall angle greater than the indenter geometry clearly indicates that the theoretical assumption is valid. (**Figure 1**). Specifically, it is assumed that the indenter tip is the portion of the tool last in contact with the specimen. This work has confirmed that assumption. However, the walls are not perfectly straight or flat and present a curved aspect in the cross section (**Figure 3**). The curved surface is likely due to degree of elastic response along the side of the indenter tool. Additionally, there was no evidence of pile up at the edge between the residual impression wall and the original material surface. These findings along with apex cracking suggest that the material does not flow as assumed in the theoretical treatment but compacts. These behaviors would be expected of a collagen-mineral micro composite [1].

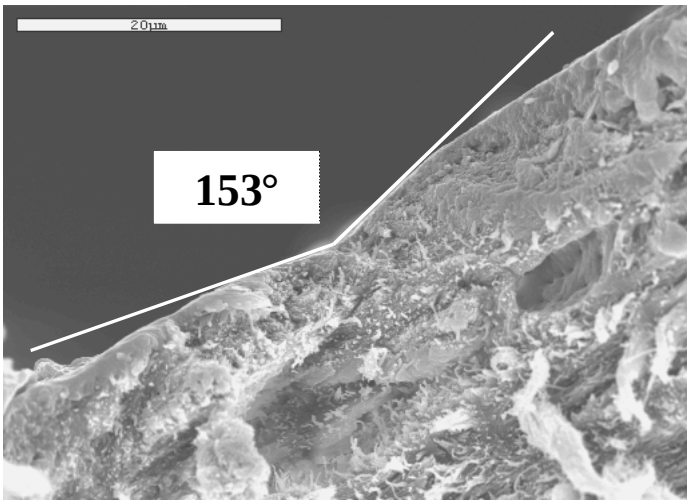

Figure 3. Typical Knoop microindentation cross section for specimen indented dry

The fact that the residual impression apex cross section angles for the wet specimen was greater than those of the dry specimen clearly suggests that the wet specimen elastically recovers more than the dry specimens. This would also be expected because the wet collagen should recover more than dry collagen much as a wet sponge would recover more than a dry one [1]. Additionally, the curved surface at the edge of the residual impression, surface unevenness, and lack of well defined boundary between the residual impression and the initial surface suggests that measuring the short Knoop residual impression diagonal with optical means is likely to be problematic. A well defined edge does not exist against which indentation machine measuring lines can be matched. This lack of definition introduces a source of subjectivity and, therefore, measurement error. This work might well extend to nanoindentation residual impressions as well. It would be reasonable to expect that similar material behavior would be found.

It also seems reasonable to conclude that the local elastic response exhibited by the material may be different from that of the bulk material. Necessarily, indentation compacts the material leaving it locally more dense and fractured. However, it may also be, given the degree of agreement between elastic modulus determination methods of nanoindentation and macroscopic tensile tests, that the same properties are being measured. It can be argued that any strain, to which bone is subjected, will result in some degree of fracture. The question of what exactly is measured during micro or nano indentation remains unanswered. Finally, cracks at the indentation apex suggest that estimates of fracture toughness in bone may be possible employing microindentation and SEM, as has been done for ceramic materials [2].

ACKNOWLEDGEMENTS

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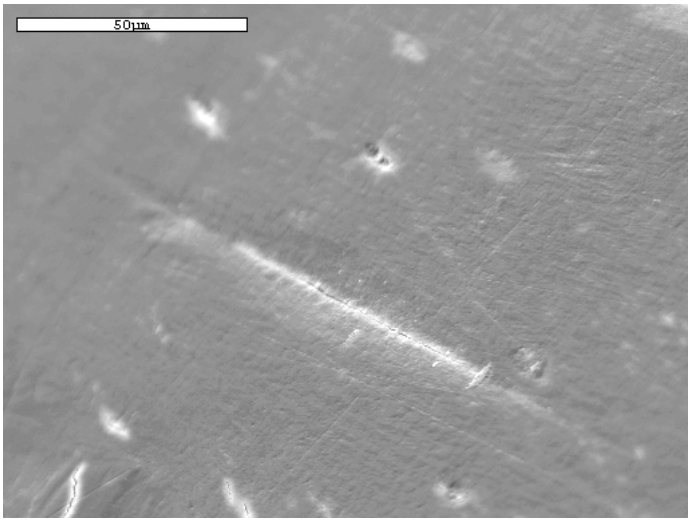

Figure 4. Crack at microindentation apex.