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Microhardness distribution of the tibial diaphysis and test site selection for reference point indentation technique

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Abstract

Indentation hardness test is a good in vitro method of bone quality assessment. The purpose of this study is to explore the distribution characteristics of bone tissue microhardness in tibial diaphysis and provide theoretical support for the test site selection of the reference point indentation technique.

Three fresh right tibias were obtained from 3 cadaver donors. The tibial diaphysis was evenly divided into 6 sections. Bone specimens with a thickness of 3 mm were cut from each part. After appropriate management, micro-indentation tests were performed in various regions of the specimens to acquire the microhardness values of the tibial diaphysis. Statistical analysis was performed by randomized block design variance analysis to study the distribution characteristics of bone microhardness.

72 regions were selected for 360 effective indentations. We found that the bone microhardness is inhomogeneous in tibia diaphysis. Mean hardness value of the anterior, medial, posterior, lateral region of tibia diaphysis was 45.58 ± 4.39 Vickers hardness (HV), 52.33 ± 3.93 HV, 54.00 ± 4.21 HV, 52.89 ± 4.44 HV, respectively. The anterior cortex exhibits lower microhardness value than the other regions ($P < .001$). Within the same region, microhardness varies significantly with positions in the tibial diaphysis. The variations in indentation hardness are bound to have a significant impact on the comparability of different reference point indentation (RPI) studies.

The results of this study indicated the regional microhardness difference in the human tibia diaphysis. The microhardness of different planes in the same region is also inconsistent. Inhomogeneous distribution of indentation microhardness would have considerable influence in the test site selection of RPI technique. The data collected in our study would contribute to the design of highly precise 3D printing implants and bionic bones with gradient elastic modulus.

Abbreviations: BMD = bone mineral density, DXA = dual energy x-ray absorptiometry, HV = Vickers hardness, RPI = reference point indentation, SPSS = statistical package for the social science.

Keywords: cortical bone, microhardness, microindentation, reference point indentation, tibia

1. Introduction

Bone is an anisotropic and inhomogeneous composite and it has an ordered structure. The ability of bone to resist fracture is often referred to as "bone strength." The higher the bone strength, the less likely it is to have a fracture.[1] It is agreed that around 60% of the bone's mechanical competence could be explained by bone mineral density (BMD) derived by quantitative computed tomography (DXA) or quantitative computed tomography.[2] Bone quality is the remainder part. The assessment of bone quality requires invasive procedures. Indentation technique is an important methodology for the evaluation of bone mechanical properties and was firstly reported in 1966.[3,4] It is a nondestructive test and allows for repeated examinations of small and awkward structures. Therefore, the indentation technique is well-suited for examining local mechanical properties in inhomogeneous bone material.[5] Hardness, the result of indentation test, is largely influenced by the mineralization process of bone,[4] and is a fairly good predictor of Young's modulus and yield stress in physiological condition.[6] However, this technology is impractical clinically, due to its high demand for test samples.

Reference point indentation (RPI) technique was first reported in 2006.[7] It is a new indentation technique that permits in vivo measurements of bone material properties in humans.[8] RPI instruments perform micro-indentation test by inserting a probe at tibia surface, applying several cycles, the indent distance was considered to reflect mechanical properties of bone tissue. Since its invention, the RPI technique has been used to identify bone quality declines due to various causes, such as aging,[9] type 2 diabetes,[10] glucocorticoid-induced osteoporosis,[11] Paget's disease.[12] Bone material strength index which is the output of the reference point indentation technique is believed to be an assessment of bone quality beyond the results of DXA.[13]

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Previous researches believed that bone tissue hardness obtained by indentation technique was on average homogeneous in long bone diaphysis.[14,15] Based on this view, the selection of RPI test sites is not strictly defined. It is generally described as a flat surface/anterior surface of mid-tibia,[16,17] or midpoint from the proximal end of the tibial plateau to the distal edge of the medial malleolus.[10] However, a recent study[18] found that RPI values varied significantly with test sites, even within the same bone. The selection of different test sites in each study also hinders the comparability between studies.

To address these issues, detailed microhardness distribution of 3 fresh tibia diaphysis was measured using the microindentation technique. Our hypothesis is that the hardness distribution of bone tissue in tibia diaphysis is not uniform, and the uneven distribution has a significant impact on RPI results. The result of our study would provide a theoretical basis for the test site selection of RPI technique.

2. Material and methods

2.1. Sample preparation

Three human skeletons (Chinese from Hebei province) were obtained from the Anatomy Department of Hebei Medical University (Shijiazhuang, Hebei, China). The study was approved by the ethics committee of the third hospital of Hebei Medical University and registered on the WHO International Clinical Trials Registry Platform. Three right tibias were obtained from 3 cadaver donors. The 3 donors' age is 62 (male, donor a), 45 (female, donor b), and 58 (male, donor c), respectively. All 3 skeletons were examined by X-ray and CT to exclude skeletal pathology. All the bones were freshly harvested and soft tissues were removed within 12 hours, then wrapped in wet gauze, stored in plastic bags at −20°C until the beginning of the sample preparation and in between the procedure steps.

Preliminarily, according to AO principle, the tibia was divided into 3 parts, the proximal, diaphysis and the distal. Tibia diaphysis was sawed by a 10′ band saw equally into 6 segments to facilitate further precision cuts. Precision cuts were made with a Buehler Isomet 11-1280-250 low speed diamond saw (Buehler Ltd., Lake Bluff, IL). Specimens with a thickness of 3 mm were cut from each part of bones in a direction perpendicular to the anatomic axis of tibia. The sampling position in tibia diaphysis is shown in Figure 1A. After being fixed on glass sheets with epoxy resin, the specimens were polished using sandpaper, switching progressively to finer sandpaper down to 2000 grit. A constant stream of water was used to cool the samples during all cutting and polishing operations. Once the polishing procedure was

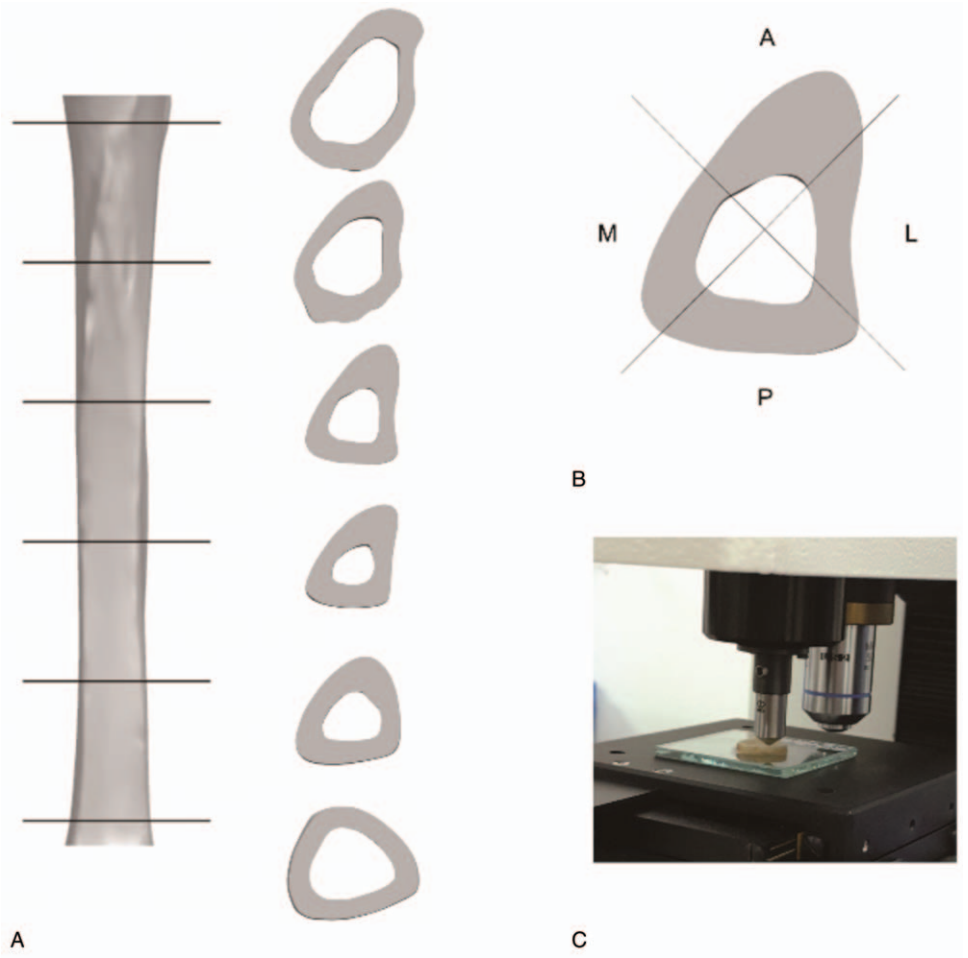

Figure 1. Procedure of microhardness measurement. (A) Sampling location in tibial diaphysis and the bone specimens. (B) Regions division of bone specimen. A = anterior region, c = microhardness measurement, L = lateral region, M = medial region, P = posterior region.

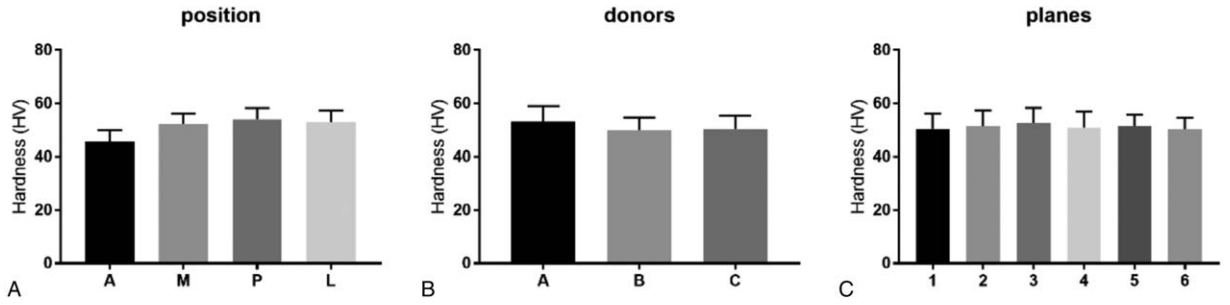

Figure 2. Comparison of microhardness values. (A) Mean microhardness values in different regions. (B) Mean microhardness values in each donor. (C) Mean microhardness values in different planes of tibial diaphysis.

completed, the samples were wrapped with wet gauze and stored in plastic bags at −20°C.

2.2. Microindentation test

Each specimen was divided into 4 regions: anterior, medial, posterior, and lateral, marked respectively with A, P, M, and L. The regional division can be seen in Figure 1B. Microindentation (Vickers testing) was performed using a microhardness tester (Model KB5BVZ-Video, Germany) with a Vickers diamond indenter, and the units were measured as Vickers hardness (HV) or kgf/mm^2. Before each of the experiments, all samples used were immersed in Ringer's solution for 0.5 hours to assure the rehydration of the bone tissue.[19,20] Before indentation, it was verified macroscopically that the location is far from microfracture and bone surface was intact. Five effective indentations were performed in each region. Indentations were carefully made (Fig. 1C) with a distance of at least 5 times the length of diagonals from each other to avoid any deformation of neighboring indentations. According to the literature,[6,21] indentations in which one diagonal was 10% longer or more than the other were ignored.

According to the standard test method from American Society for Testing Materials and previous studies,[19,22,23] the procedure was defined by a load of 50 gf; the indentation time was set to 50 seconds and dwell time was set to 12 seconds. Hardness value (HV/0.05) was computed for each indentation.

2.3. Statistical analysis

The statistical analysis aimed to determine differences among the hardness values measured in different regions, anatomic sites and donors. Mean values and associated standard deviation are shown for normally distributed data. Otherwise median values and interquartile range were given. Microhardness values were compared across sites in each donor by randomized block design analysis of variance followed by Tukey post hoc tests. Normality was tested using the Shapiro–Wilk test. Alpha was set to $P < .05$ and considered statistically significant.

3. Results

Totally 360 effective indentations were made in 72 regions. General distribution of bone microhardness in tibia diaphysis is shown in Figure 2 and Table 1. As hypothesized, the bone microhardness is inhomogeneous among different anatomic sites of tibial diaphysis. Microhardness value ranged from 36.50 HV to 65.40 HV, with a mean of 51.20 ± 5.37 HV.

Mean microhardness value of the 1 to 6 plane in tibia diaphysis is 50.41 ± 5.66 HV, 51.42 ± 5.92 HV, 52.71 ± 5.57 HV, 50.92 ± 5.96 HV, 51.88 ± 4.72 HV, 50.22 ± 4.45 HV, respectively (Fig. 2C). However, there is no statistical difference among the microhardness values of the 6 planes ($P > .05$).

Mean hardness value of the anterior, medial, posterior, lateral region of tibia diaphysis is 45.58 ± 4.39 HV, 52.33 ± 3.93 HV, 54.00 ± 4.21 HV, 52.89 ± 4.44 HV, respectively (Fig. 2A). Among the 4 regions, the differences in microhardness value are statistically significant ($P < .001$). As shown in Figure 3A, the hardness value in the anterior region is the lowest. Posterior region is the hardest part. Mean microhardness value of the 3 donors is 53.20 ± 5.77 HV, 50.00 ± 4.72 HV, 50.41 ± 5.02 HV, respectively (Fig. 2B). Differences among the 3 donors are statistically significant ($P < .001$). Data and post hoc test results could be seen in Table 2.

Table 1

Mean value of microhardness for each region in different planes of the tibia.

Plane	A Mean (±SD)	M Mean (±SD)	P Mean (±SD)	L Mean (±SD)
1	43.78 (2.94) ^{Aa}	49.68 (3.78) ^{Ab}	53.65 (4.15) ^{Ac}	54.54 (4.21) ^{Ac}
2	44.15 (3.04) ^{Aa}	54.14 (3.72) ^{Bb}	54.72 (4.45) ^{Ab}	52.69 (5.16) ^{Ab}
3	47.53 (5.34) ^{Aa}	54.59 (4.12) ^{Bb}	54.27 (5.07) ^{Ab}	54.47 (4.57) ^{Ab}
4	45.20 (6.45) ^{Aa}	52.05 (4.05) ^{ABb}	53.99 (4.03) ^{Ab}	52.46 (5.13) ^{Ab}
5	46.79 (3.67) ^{Aa}	51.83 (3.30) ^{ABb}	54.71 (2.15) ^{Ab}	52.74 (3.01) ^{Ab}
6	46.06 (3.02) ^{Aa}	51.68 (2.92) ^{ABb}	52.67 (5.11) ^{Ab}	50.45 (3.52) ^{Ab}

Means and standard deviation followed by different lowercase letters in 1 same line and different uppercase letters in one same column indicate statistically significant difference for randomized block design ANOVA, followed by Tukey post hoc Test (5% significance level).

A = anterior region of the tibia, L = lateral region of the tibia, M = medial region of the tibia, P = posterior region of the tibia, SD = standard deviation.

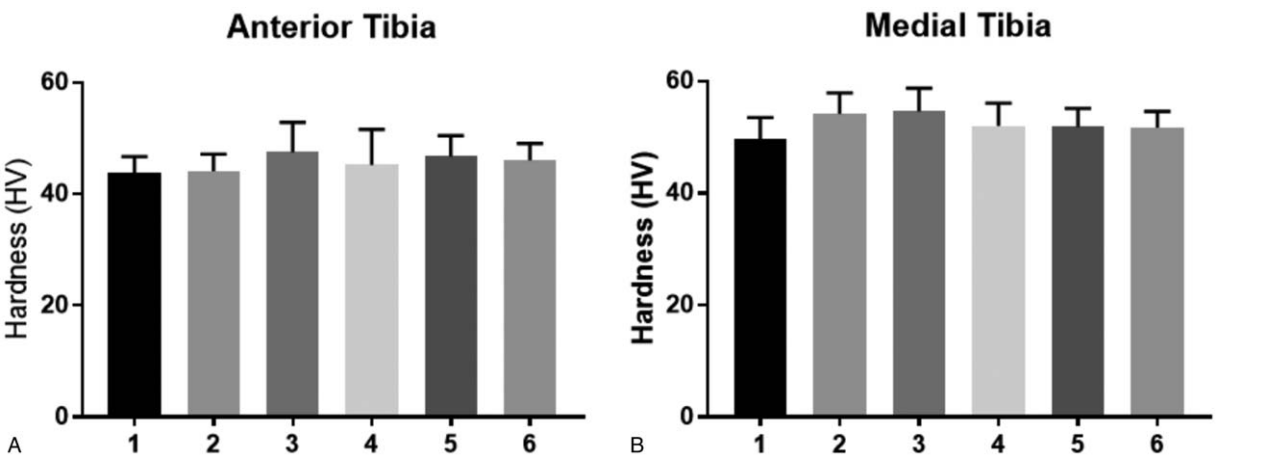

Figure 3. (A) Mean microhardness values of different planes in the anterior region of tibial diaphysis. (B) Mean microhardness values of different planes in the medial region of tibial diaphysis.

In the anterior region of the tibia, plane 3 has the maximum microhardness value while plane 1 has the minimum value. Differences among the 6 planes are not statistically significant ($P > .05$). In the medial region of the tibia, plane 3 has the maximum microhardness value while plane 1 has the minimum value. Differences among the 6 planes are statistically significant ($P < .01$). Data and post hoc test results could be seen in Table 1.

4. Discussion

The present study aimed to investigate whether the bone indentation hardness is uniform in the tibial diaphysis. The microhardness of cortical bone tissue from 3 tibial diaphyses was studied. Traditionally, it is believed that cortical bones exhibited higher indentation hardness than trabecular bones; but within the same tissue type, hardness was generally thought to be homogeneous.[14,24] Different from previous researches, we found that the bone microhardness is inhomogeneous in different regions of the tibia shaft. The microhardness value in the posterior region was the highest, whereas microhardness in the anterior region was the lowest.

In physiological conditions, the microhardness is mainly determined by the mineralization process of bone tissue, especially the secondary mineralization.[4] The higher mineralization tissue exhibited higher microhardness value. The process of mineralization is regulated by both genetic factors and local loading history.[25,26] In high strain/stress areas, the bone modeling process is activated. New bone begins to form, and the mineral content of bone is increased. Thus the bone becomes stiffer to reduce the abnormally increased strain. The process is called the functional adaption.[27] In the present study, there was no statistically significant difference in the microhardness of tibial diaphysis in different planes ($P > .05$). The mean microhardness value of each plane ranged from 50.22 to 52.71 HV. These results suggested that the tibia diaphysis was subjected to similar stress at different levels during daily activities.

The indentation microhardness measured on the different regions showed a statistically significant difference ($P < .001$). The anterior cortex had the lowest microhardness (45.58 ± 4.39 HV), significantly lower than the medial cortex (52.33 ± 3.93 HV), posterior cortex (54.00 ± 4.21 HV), and lateral cortex (52.89 ± 4.44 HV). This variation may be related to the load bearing mode of tibial diaphysis. It is believed that the mechanical axis of tibial diaphysis is closer to the posterior cortex in the sagittal plane.[28] It could be speculated that there are more axial loads which are subjected to the posterior cortex in daily activities. Not only that, a recent study by Yang et al found that,[29] the tibia bends to posterior during walking and running. The posterior cortex experienced higher compressive loading than the anterior cortex during the gait cycles. This result is consistent with previous research, Lai et al[30] found that the posterior cortex of tibia diaphysis had higher volumetric BMD than the anterior, medial and lateral cortex. They also thought this is a result of mechanical adaption.

Indentation technique has been used to monitor the mechanical property changes in cortical bones at the tissue level. It can track the very early stage of bone strength degeneration in patients exposed to systematic glucocorticoid treatment.[11] At this stage, there is no alteration of BMD imaging by DXA. There is no universally accepted standard for the measurement site selection

Table 2

Microhardness value data and results of the Tukey post-hoc test.

Region	n	Mean	SD	Donor	n	Mean	SD
A	90	45.58^{a}	4.39	a	120	53.20^{a}	5.77
M	90	52.33^{b}	3.93	b	120	50.00^{b}	4.72
P	90	54.00^{c}	4.21	c	120	50.41^{b}	5.02
L	90	52.89^{bc}	4.44				

Groups with the same subscripts are not significantly different ($P > .05$). A = anterior region of the tibia, L = lateral region of the tibia, M = medial region of the tibia, P = posterior region of the tibia.

of RPI technique. Coutts et al[18] studied the in vitro indentation of human cadaveric femurs, found that RPI values are highly influenced by the test site. However, the femur is not a common site for RPI measurement. We measured the microhardness of tibia cortex in different regions. There is a statistically significant difference of indentation microhardness between the anterior and medial cortex of tibial diaphysis ($P < .001$) (Table 2). Within the anterior cortexes of the tibial diaphysis, plane 3 had the highest microhardness value (Fig. 3A). However, the differences among the planes are not statistically significant ($P > .05$). There are statistically significant differences among the planes of medial cortexes. Plane 2 and 3 had the highest microhardness values, significantly higher than the plane 1 (Fig. 3B). The indentation microhardness varies significantly with the measurement sites. Thus, it is important to perform the indentions in close proximity to obtain more accurate results. From the discussion above, we can speculate that studies that select different measurement sites (anterior or medial cortex of the tibia) are less comparable. There are statistically significant differences in microhardness among the 3 donors, but the value does not differ greatly (Table 2). Such inter-individual differences may have a certain influence on the comparison between different groups of people.

Based on the results of this study, we have 2 recommendations for test site selection of RPI technique. First, since microhardness of the anterior and medial cortex of tibia is not the same, and the microhardness of different planes in the same region is also inconsistent, the test sites should be selected in the same region, in close proximity to obtain precise results. Second, the microhardness value varies with different individuals, the variation should be considered in the experimental design.

In the past decade, three-dimensional (3D) printing technology provides the ability to construct highly customizable implants to improve patient outcomes.[31] 3D printed bone grafts provide a new level of anatomical precision for bone defect reconstruction. However, 3D printed bone grafts so far are homogenous. When homogeneous implants are exposed to the complex mechanical environment, microdamage will accumulate in the high strain area. Bone microhardness has an almost linear relationship with Young's modulus.[32] The data collected in our study would contribute to the design of highly precise 3D printing implants, which are consistent with the human skeleton in Young's modulus.

The present study has some limitations. First, the number of tibias used in the study was small; however, the difference of indentation hardness among different regions is statistically significant despite the small numbers. Second, although indentation technology is used in both Vicker's microhardness and RPI measurement, there has not been any identified relationship between results of the 2 techniques. The conclusions must be considered as preliminary results. Third, microhardness technology is difficult to apply to clinical practice, which limits its development.

In conclusion, the results of this study indicated the regional microhardness difference in the human tibia diaphysis. The anterior cortex had the lowest microhardness value, are significantly lower than the other region. The microhardness of different planes in the same region is also inconsistent. Inhomogeneous distribution of indentation microhardness would have considerable influence in the test site selection of RPI technique. The data collected in our study would contribute to the design of highly precise 3D printing implants and bionic bones with gradient elastic modulus.

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