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From the grave to the lab: evaluation of archaeological human bone preservation based on micro-computed tomography analysis

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Abstract

Timely understanding of the preservation status of archaeological human bones is the foundation for conducting scientific archaeological work. This paper applies Micro-CT technology to analyze the microscopic preservation status of ancient human femora unearthed from the Rui State site in Liangdai Village, Hancheng, Shaanxi, the Chejiasi Cemetery in Baoji, and the Ouerping Cemetery in Xizhou Village, Yushe, Shanxi, and obtained imaging results and cortical bone porosity (Ct.Po), bone volume fraction (BV/TV), and bone density (BMD) parameters for 9 samples. The results show that the poorly preserved fragile group has lower Ct.Po, BV/TV, and BMD, and macroscopically presents fragile and porous features; while the well-preserved dense group has relatively higher Ct.Po, BV/TV, and BMD, and macroscopically appears solid and dense. This study employs Micro-CT technology to analyze the micro-preservation status of human bones from historical periods, confirming the effectiveness of this technology in revealing the microstructure of ancient human bones, and providing a reference for establishing a human bone preservation status evaluation system.

Keywords Micro-CT, Archaeological human bone, Bone preservation, Cortical porosity, Bone volume fraction, Bone mineral density

Introduction

Archaeological human bones provide valuable anthropological physical data for studying the physical characteristics, health status, and behavioral patterns of ancient human individuals and groups. They are also key relics for exploring archaeological topics such as human origins, population integration, and cultural interaction. However, in archaeological excavation fieldwork, understanding of the preservation status of unearthed human bones largely depends on macroscopic observation and subjective judgment, lacks microscopic preservation information, and the evaluation results are easily affected by errors between different observers, making it difficult to provide scientific, comprehensive, and targeted protective treatment suggestions. Moreover, compared to other fragile cultural relics, the evaluation standards for the preservation status of archaeological human bones have not yet been formed, on-site evaluation research is relatively scarce, and microscopic studies of human bones unearthed in historical periods are rare. In addition, the sampling and operation process of traditional detection methods are cumbersome and complicated, these factors jointly restrict the systematic research and effective protection of archaeological human bones.

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The human skeletal system is complex and has unique material properties. During the long process of burial underground, various factors such as diagenesis and burial environment contribute to the gradual replacement and loss of collagen proteins within the bones [1], resulting in significant changes in mineral crystallinity, histological characteristics, and pore structure [2]. As a result, the bones undergo postmortem alteration. Currently, existing methods for assessing the archaeological human bones preservation status mainly focus on spectroscopy, histology, and pore studies. Spectroscopic methods, such as Fourier-transform infrared spectroscopy (FTIR), have been widely used to study the mineral crystallinity of bones [3–7]. By calculating the infrared splitting factor (IRSF), the inorganic component proportion of the bones can be reflected. On the microscopic scale, histological analysis can provide information on the preservation of biological remains at the early stage of burial and at the molecular level after death [8–11]. Many researchers have used optical microscopy [12–14] or scanning electron microscopy [15, 16] for qualitative evaluation of the microscopic structure of bones. However, there are subjective differences in image interpretation, and the resin embedding method used for bone samples can be destructive. Additionally, during the burial process, bones are influenced by processes such as biodegradation and mineral dissolution, leading to changes in porosity, typically manifested as a decrease in microporosity ('s' porosity), an increase in macroporosity ('l' porosity), and loss of bone density [17]. Studies have shown that the porosity in bone microstructure is correlated with collagen content [18]. However, commonly used methods such as mercury intrusion porosimetry (HgIP) and BET N_2 adsorption have drawbacks such as complex operation, long duration, and destructive nature, even though they can provide information on pore size and microporosity.

The diagenetic alterations of archaeologically unearthed human bones present significant challenges to bioarchaeological research, including isotopic and trace element analyses. Scientific assessment of the preservation status of archaeological bones is not only a crucial aspect of on-site conservation but also a preliminary screening step for bioarchaeological research. Identifying the preservation status of ancient bone samples and selecting those that are uncontaminated or minimally contaminated is a prerequisite for archaeometry research. Traditional structural detection techniques introduce a significant amount of distortion, which interferes with accurate identification of pathological conditions in archaeological human remains [19]. Therefore, there is a need to find a method that can quickly and accurately obtain microscale preservation indicators of

the internal structure of bones in a non-destructive or minimally destructive manner. This approach is of considerable practical significance for advancing laboratory research in bioarchaeology and for the effective conservation of archaeological bones.

Micro-computed tomography (Micro-CT) is a non-destructive three-dimensional imaging technique that has been extensively applied across various fields, including biomedicine and materials science. This technology facilitates high-resolution, non-invasive scanning of samples, enabling the precise capture of internal microstructures and their parameters without compromising the integrity of the sample's internal architecture. Compared to traditional methods such as quantitative microimaging and scanning electron microscopy, which are conventionally utilized to assess the degree of mineralization, Micro-CT demonstrates significant advantages [20]. Furthermore, the accuracy and efficacy of Micro-CT in the field of scientific archaeology, particularly in the study of skeletal microstructures, have been corroborated through numerous scientific research studies [21–26]. In contrast to contemporary X-ray photography techniques [27] and traditional CT technologies employed in the realm of cultural heritage preservation research, Micro-CT boasts the advantage of high-resolution capabilities at the micrometer scale [23], accurately reflecting the internal microstructural characteristics of bones. When juxtaposed with the two-dimensional histological section analysis, Micro-CT allows for virtual continuous slicing, reconstruction of samples, and the construction of three-dimensional models. This enables the rotation of the three-dimensional images for observation from any angle, and the results obtained from both methods exhibit a high degree of consistency in terms of bone tissue structure and bone density [28].

The establishment of a scientific and systematic evaluation method is crucial for timely understanding and preserving archaeological human bones. A key step in the feasibility exploration of on-site assessment research on the preservation status of unearthed human bones is to establish evaluation indicators that reflect the micro-preservation condition of the bones. Therefore, this study employs Micro-CT technology to conduct imaging analysis and parameter calculation on nine ancient human femur samples from three different locations. By analyzing three parameters—cortical porosity (Ct.Po), bone volume fraction (BV/TV), and bone mineral density (BMD)—the study identifies the micro-preservation characteristics of the samples and validates the effectiveness and accuracy of the selected micro-indicators. The findings provide significant reference for the standardization of future assessments of the preservation status of culturally significant archaeological human remains.

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Materials and methods

Sample profile

The femur, as a commonly used sample in biomechanical research, is an ideal specimen for reflecting skeletal structural composition due to its distinct cortical and trabecular bone composition. Studies have indicated that the degree of degradation and diagenesis of archaeologically excavated bones is influenced by the burial environment [29]. To eliminate the randomness of results from a single location, this study selected nine ancient human femur samples from the following archaeological sites: the Wei State site at Liangdai Village in Hancheng, Shaanxi; the Chejiasi Cemetery in Baoji, Shaanxi; and the Warring States period cemetery at Ouerping, Yushe County, Shanxi.

These samples, despite being from different historical periods and exhibiting varying macroscopic preservation states, are all from similar burial environments. Geographically, both the Liangdai Village Wei State site and the Chejiasi Cemetery are located in the Guanzhong Basin of Shaanxi, near the Yellow River and the Wei River, respectively. The Ouerping Cemetery is situated in the Jinzhong Basin of Shanxi, close to the northern source of the Zeng River. All three locations have a warm temperate continental monsoon climate, with soil types predominantly consisting of brown earth.

Prior to Micro-CT scanning of the samples, to verify the reliability and accuracy of Micro-CT, we conducted macroscopic descriptive observations and measurements of average surface hardness on the nine samples. This approach allowed us to compare the results of traditional macroscopic analysis with those of Micro-CT's micro-evaluation of the preservation status. Considering the portability and rapidity required for on-site archaeological assessments, the surface hardness measurement of unearthed human bones was conducted using a D-type digital Shore hardness tester (Fig. 1). This device offers a non-destructive and efficient method to measure the hardness of the bone surface, that can be immediately compared to the micro-evaluation results obtained from Micro-CT scans. To ensure consistency and reliability of the results, this study selected three points on the femoral surface, 10 cm away from the nutrient foramen, and measured uniformly from the proximal to the distal end. The readings were recorded, and the average hardness value was calculated.

In the present experiment, the macroscopic descriptive observations and surface hardness measurement data for the nine archaeologically excavated human femur samples are summarized as follows (Table 1):

The data provided offer a detailed account of the macroscopic preservation conditions of each sample, which is essential for the subsequent micro-CT analysis and

Fig. 1 D-Type digital Shore hardness tester operation

discussion of bone preservation assessments. By integrating macroscopic and microstructural data, we can further validate the effectiveness of Micro-CT in evaluating the preservation status of archaeological human bones.

Furthermore, the study's sample selection excluded all femora that showed pathological changes, ensuring that the chosen samples were from healthy individuals.

Table 2 presents the sex and age at death for 9 samples, which allows for the inclusion of individual anthropological information in the discussion of results. These data are based on methods of sex and age estimation in physical anthropology, such as the examination of the pubic symphysis, auricular surface, dental attrition, cranial suture closure, sternal rib ends, and other indicators. This comprehensive approach enables a more thorough understanding of the samples and helps to distinguish between preservation changes associated with physiological aging and those related to burial conditions. By integrating this information, this study providing a clearer context for the exploration of the micro-preservation status and micro-features of ancient human bones.

These samples, with their varying conditions, provide a comprehensive range of bone preservation statuses for the study, allowing for a detailed analysis of the micro-preservation characteristics.

Although Micro-CT technology enables non-destructive scanning of samples, the specimens selected for this study were ancient human femora, which are relatively large in size and present challenges due to the limited capacity of the sample chamber. Consequently, direct whole-bone scanning was not feasible. To address this issue and meet the research requirements, the samples were prepared through a process of sectioning. For each specimen, a Sect. 10 mm below the nutrient foramen of the femur was chosen, and a steel saw was used to physically cut a 10 mm length

Table 1 Information for ancient human femur samples

Number	Sites	Tombs number	Macro descriptions	Average surface hardness (HD)
LDR490	Liangdai Village Rui State site	M2	Exhibited a porous and brittle bone surface, prone to fracture and breaking during sectioning	5.00
LDR521		M6	Showed a mottled and moldy bone surface with visible cracks and flaking bone quality	5.83
LDR520		M298	Had cracks on the bone surface with flaking bone quality	6.17
LDR489		M529	Presented with a porous and brittle bone surface, mottled with mold spots, and was also prone to fracture during cutting	5.33
LDR522		M1413	Had severe surface erosion and high mineralization, with extremely brittle bone quality that broke easily upon sectioning	5.17
BJC487	Chejiasi Cemetery	M3	With hard bone quality	6.50
BJC486		M46	With a relatively porous and moldy bone surface	4.67
SXP488	Ouerping Cemetery	2017YOT1304⑥	Displayed smooth bone surfaces and hard bone quality	6.83
SXP491		2018YOT1205③	Displayed smooth bone surfaces and hard bone quality	6.33

Table 2 Sex and age at death of 9 samples

Number	Sex	Age at death
LDR490	Female	35
LDR521	Female	30
LDR520	Male	29–30
LDR489	Female	≥ 56
LDR522	Female	18–23
BJC486	Female	18–23
BJC487	Male	≥ 56
SXP488	Male	24–35
SXP491	Male	24–35

for scanning as the sample block. Additionally, to accurately reflect the preservation status of human bones unearthed at archaeological sites, the samples were not subjected to any chemical pretreatment. Figure 2 illustrates an example of the sampling site and the scanned sample block. After preparation, imaging and analysis were conducted on designated areas of cortical and trabecular bone for the aforementioned nine samples.

This methodological approach ensures that the samples are representative of the original bone condition, allowing for a detailed examination of the microstructural features without altering the natural state of the archaeological remains. The absence of chemical pretreatment maintains the integrity of the samples, providing a more authentic basis for the assessment of bone preservation in an archaeological context. The resulting Micro-CT scans offer a valuable insight into the micro-preservation characteristics of the ancient femurs, which are instrumental in better understanding the preservation status of human skeletal remains unearthed at archaeological sites.

Micro-CT analysis parameters

In the field of biomedicine, Micro-CT scanning technology is extensively utilized for the analysis of skeletal structures, with research typically focusing on two main categories: cortical bone and trabecular bone. Common parameters used to describe cortical bone structure include cortical thickness (Ct.Th), cortical porosity (Ct.Po), and cortical bone area (Ct.Ar). On the other hand, parameters frequently used to describe trabecular bone structure encompass trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), and bone volume fraction (BV/TV).

Given the differences in preservation conditions between archaeologically excavated human bones and modern skeletons, this study has selected cortical porosity (Ct.Po), bone volume fraction (BV/TV), and bone mineral density (BMD) as analytical indicators for assessing the micro-preservation status of unearthed human bones. This selection is tailored to meet the practical needs of the assessment research.

Cortical porosity

Cortical porosity (Ct.Po) is an indicator of the degree of porosity within the cortical bone, which can reflect the extent of degradation and the overall structural integrity of the bone. These porosities can determine the elasticity and ultimate strength of bone tissue [30]. Compared to fresh bones, archaeological bones often exhibit relatively higher porosity due to the increased volume of pores, which accelerates the dissolution of minerals and the degradation of collagen. Numerous studies have confirmed that the porosity within the bone is a significant indicator of the degree of diagenesis [17, 18, 31–33], revealing the micro-level changes that bones undergo during the burial process.
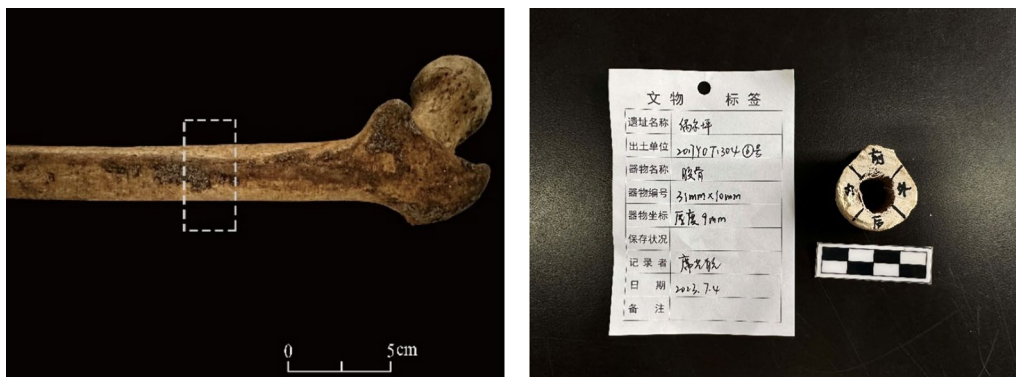
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Fig. 2 Sample sampling site example and warehouse scanning sample block example

In comparison to other diagenetic parameters, porosity provides a comprehensive reflection of the changes in the inorganic and organic components within the bone, as well as the macroscopic and microscopic evolution of bone structure. As such, it emerges as a key parameter in assessing the preservation status of bones.

Bone volume fraction

The bone volume fraction (BV/TV) represents the proportion of bone volume within the total tissue volume and is a critical measure of bone density and overall bone mass. BV/TV which stands for the ratio of trabecular bone volume (BV) to total volume (TV) within a region of interest, is a crucial parameter for describing the microstructure of trabecular bone. This metric provides a quantitative assessment of the relative amount of bone tissue compared to the total volume of the specimen, including both bone and marrow spaces.

BV/TV is significant for several reasons. It serves as a measure of bone mass, which is essential for understanding the overall health and strength of the skeletal system. This parameter is particularly useful in assessing both cortical and trabecular bone, as it provides insight into the density of the bone tissue. Overall, BV/TV is a critical parameter in the analysis of trabecular bone microstructure, offering a wealth of information that can be applied across various disciplines, from clinical research to the study of ancient human remains. Its utility in assessing bone quantity and quality makes it an indispensable tool in the field of bone biology and osteoarchaeology.

Bone mineral density

Bone mineral density (BMD), which indicates the amount of minerals in a given area of the skeleton, is a widely recognized indicator of bone strength. BMD providing insights into the mineral content of the bone and its susceptibility to fractures and other pathological conditions.

The assessment of BMD is particularly important in the diagnosis and management of osteoporosis, as it allows healthcare professionals to evaluate an individual's risk for developing the condition and to monitor the effectiveness of treatment strategies. In the context of osteoporosis, a lower BMD is associated with bones that are more porous and fragile, which are more susceptible to breakage under stress or strain.

In addition to its clinical applications, BMD is also a valuable metric in osteoarchaeology. For a long time, BMD has been considered a key factor affecting the quantity of bone remains unearthed from tombs [33]. There is a strong correlation between bone mass and bone strength; a reduction in BMD implies a weakening of bone strength and an increased risk of fractures [34].

Overall, BMD serves as an indispensable parameter in both clinical practice and research settings. It is a critical measure that reflects the strength of the skeletal system and acts as a prognostic factor for the risk of fractures. BMD quantifies the amount of bone mineral content per unit area of bone, providing a snapshot of an individual's bone preservation.

By focusing on these specific parameters, the study aims to provide a comprehensive evaluation of the micro-preservation status of archaeological human bones, taking into account the unique challenges and requirements associated with the analysis of ancient skeletal remains. This approach ensures that the findings are relevant and applicable to the field of archaeological science, contributing to a better understanding of the factors that influence the long-term preservation of human bones in various burial environments.

Experimental methods
Micro-CT scanning parameters

The experiment utilized the Micro-CT VIVA 80 scanning system (CT80, Scanco Medical, Switzerland) from the

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School of Life Sciences at Northwestern Polytechnical University. The samples were vertically scanned within a cylindrical holder measuring 45 mm in diameter and 100 mm in height. The scanning parameters were set at a voltage of 70 kV and a current of 113 μA, with an exposure time of 152 ms for each step. All samples were scanned at a resolution of 5 μm (isotropic voxel size), and a 0.5 mm aluminum filter was used to attenuate the X-ray beam.

To prevent any potential impact on the skeletal parameters from physical sectioning during secondary sampling, the experiment opted for manual selection of a virtual 5 mm thick midsection of the samples for analysis and reconstruction during the pre-scanning phase of the Micro-CT. Each sample took approximately 25 min to scan, yielding approximately 1309 continuous tomographic images.

Sample reconstruction

After the scanning process was completed, the software Scanco Evaluation (Scanco Medical, Switzerland) was utilized to delineate regions of interest (ROIs) and reconstruct the Micro-CT imaging results. The purpose of this step was to perform quantitative analysis of the images to obtain parameters that reflect the micro-preservation of the samples. Prior to conducting morphometric quantitative detection, it was essential to ensure strict consistency in all scanning, reconstruction, and segmentation parameters.

For the analysis of Ct. Po, ROIs were selected in the bone unit area located between the outer and inner circumferential layers of the cortex for analysis. Given the variability in corrosion and mineralization degrees among the samples, the identifiable parts of the trabecular bone differed. Therefore, BV/TV and BMD were analyzed by selecting ROIs of the same area along the contour of the trabecular bone near the linea aspera in each sample, while taking care to exclude interference from rock and soil fragments within the bone. For each sample, 100 continuous slices were chosen for reconstruction, with a reconstructed thickness of approximately 0.5 mm.

After the ROIs were defined, a Gaussian filter (sigma = 0.8 voxels) was applied to minimize noise in the images. For the cortical bone ROI, a threshold range of 200–555 (increment of 10) was used to segment the bone from the background; for the trabecular bone ROI, a threshold range of 250–1000 (increment of 10) was applied for the same purpose. To ensure consistency and comparability of the experimental results, all nine samples were analyzed using the same threshold for parameter calculation. The Scanco Evaluation software's Cortical Bone script was used to calculate parameters for the cortical bone ROIs, while the Trabecular Bone script was employed for the trabecular bone ROIs. At each threshold, the Scanco software was used to calculate and record the data for Ct. Po, BV/TV, and BMD within the ROIs.

This systematic approach to image analysis and parameter calculation ensures that the results obtained from the Micro-CT scans are accurate and reliable, providing valuable quantitative data on the microstructural preservation of the archaeological bone samples. The use of consistent methods and parameters across all samples is crucial for valid comparisons and meaningful interpretations of the data in the context of archaeological research.

Results and discussion
Analysis of Micro-CT imaging results

The Micro-CT imaging results revealed significant differences in the preservation status among the nine samples. The bones that were well-preserved exhibited relatively uniform structural characteristics, indicating minimal disruption to their original microarchitecture. In contrast, bones with lower collagen content and higher degrees of mineralization appeared more porous, a phenomenon that may be associated with the increased porosity resulting from diagenetic processes [2, 18].

The Micro-CT images of the five samples from the Liangdai Village Rui State site have revealed distinct microstructural characteristics for each specimen. As shown in Fig. 3, LDR490 and LDR489 exhibit macroscopic fragility and a propensity to fracture easily, with the micrographs further demonstrating significant porosity in the cortical bone regions of these two samples, increasing from the inside out. LDR490 appears even more porous and spongy than LDR489, with darker cortical regions, indicating a higher degree of erosion. LDR520 shows several holes of varying sizes around the medullary cavity, but its cortical bone is denser compared to LDR490 and LDR489. The cortical bone of LDR521 and LDR522 is relatively dense with a few large pores.

It is also observable that LDR490 and LDR489 are distinctly encircled by a bright and dense mineralized ring on their outermost periphery, while LDR521 and LDR520 both exhibit significant fractures without having broken apart. The images presented in Fig. 4 correspond to the four samples from the Chejiasi Cemetery and the Ouerping Cemetery. Among the two samples from the Chejiasi Cemetery, BJC486 shows imaging results similar to LDR489 and LDR490 but with fewer cortical holes and less erosion. A dark area on the right side of the cortical bone in BJC486 indicates possible localized erosion. In contrast, BJC487's imaging characteristics are closer to those of LDR521 and LDR520, showing a few large pores but no significant fractures. The two samples from the
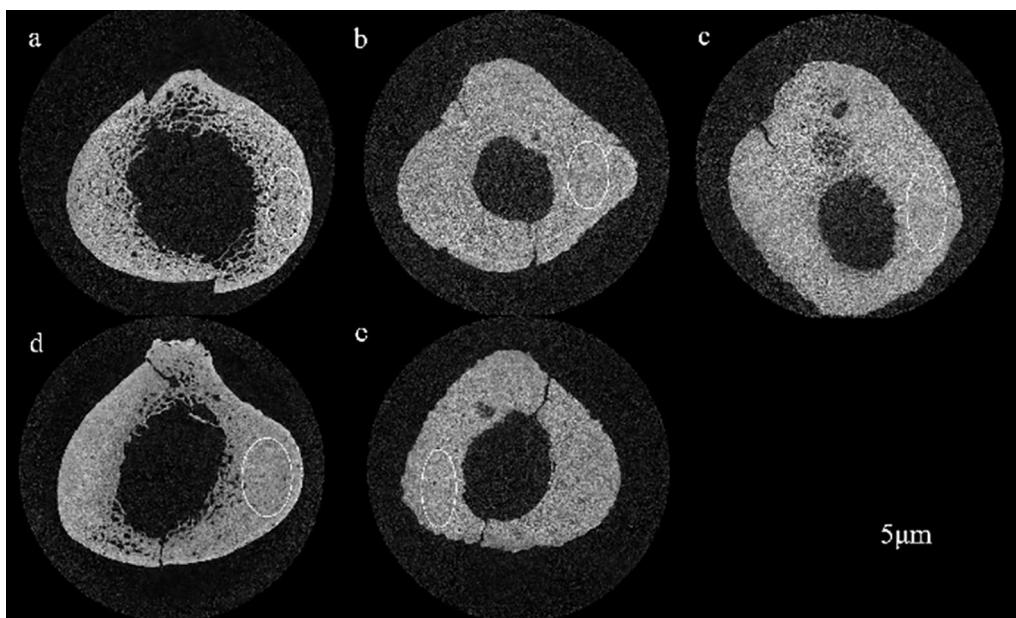
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Fig. 3 Micro-CT images of 5 samples from the Ruiguo site in Liangdai Village. From left to right, **a** LDR490; **b** LDR521; **c** LDR520; **d** LDR489; **e** LDR522
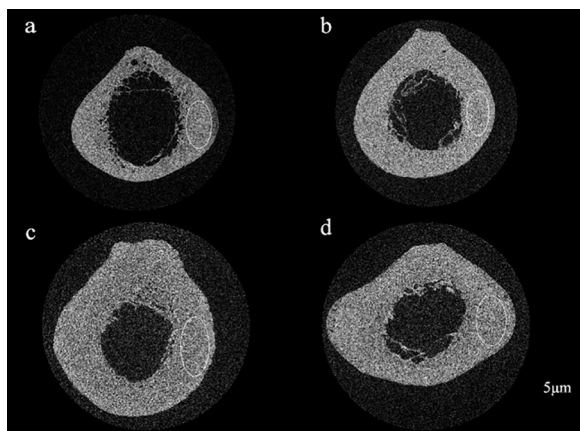

Fig. 4 Micro-CT images of the Chejiasi site and the Ouerping Site. From left to right, **a** BJC486; **b** BJC487; **c** SXP488; **d** SXP491

Table 3 Micro-CT calculation results of samples

Number	Ct.Po(%)	BMD(mg HA/ccm)	BV/TV(%)
LDR490	34.2064	125.88	21.0346
LDR521	43.2879	230.63	29.6578
LDR520	44.0850	237.04	29.0430
LDR489	32.2484	130.78	24.2867
LDR522	32.0139	174.48	24.5815
BJC486	26.9677	110.49	22.2897
BJC487	47.0466	136.74	27.7547
SXP488	48.3055	300.68	34.7325
SXP491	41.9756	262.23	31.8148

Ouerping Cemetery do not exhibit porous and spongy features. SXP488's imaging results are similar to those of BJC487, LDR521, and LDR520, with cortical bone regions all showing a denser nature. As for SXP491, it presents large pores near the external bone surface, similar to the imaging results of LDR521. Both samples have a dense structure, and no fractures were observed.

These detailed observations from the Micro-CT imaging provide a comprehensive understanding of the micro-preservation conditions of the ancient femur samples from different archaeological sites. The variations in porosity, erosion, and mineralization within the cortical bone regions are indicative of the diverse post-depositional processes these bones have undergone. The presence or absence of fractures, as well as the density of the cortical bone, are crucial factors that can influence the preservation and analysis of archaeological bone samples. Understanding these microstructural features is essential for the accurate interpretation of the health, diet, and lifestyle of the individuals to whom these bones once belonged, as well as for the broader study of human adaptation and evolution over time.

Analysis of the calculation results of Micro-CT parameters

Table 3 presents the computed microstructural parameters for five skeletal samples from the Liangdai Village Rui State site. The results show that LDR521 and LDR520 have similar values for cortical porosity (Ct.Po), bone volume fraction (BV/TV), and bone mineral density (BMD), which corresponds with the observations from Micro-CT imaging, indicating that these two samples share a similar state of preservation. LDR490, LDR489, and LDR522 also exhibit comparable Ct.Po and BV/TV values. However, LDR522's BMD is notably higher than the other two samples, suggesting lower bone strength and increased fragility, which is consistent with the macroscopic description of the sample. Compared to LDR521 and LDR520, LDR490, LDR489, and LDR522 all have lower Ct.Po, BV/TV, and BMD values, reflecting a poorer state of preservation.

For the two samples from the Chejiasi Cemetery, BJC486 and BJC487, the calculation results reveal that BJC486's microstructural parameters are closer to those of LDR490, LDR489, and LDR522, and Micro-CT imaging also shows porous characteristics in the cortical bone for these samples. In contrast, BJC487's parameters align more closely with LDR521 and LDR520, and the imaging results indicate a similar state of preservation for these samples.

The two samples from the Ouerping Cemetery, SXP488 and SXP491, have higher values for Ct.Po, BV/TV, and BMD compared to samples from the other two sites, and their overall data are similar to the results for LDR521, LDR520, and BJC487. Combining the Micro-CT imaging results, SXP488, SXP491, LDR521, LDR520, and BJC487 all exhibit denser cortical bone structures and fewer large pores, indicating a better state of preservation.

These findings underscore the importance of Micro-CT imaging and quantitative analysis in assessing the preservation status of archaeological bone samples. The consistency between the imaging observations and the calculated parameters provides a comprehensive understanding of the microstructural integrity and the potential impact of diagenetic processes on the skeletal remains.

Difference of bone microstructure calculation parameters

Based on the microstructural parameters obtained from Micro-CT scans and the imaging results, the nine samples can be categorized into two distinct groups with significant differences in preservation types: the Fragile Group and the Dense Group.

Figure 5a, b, and c illustrate the intergroup differences in microstructural parameters for these two groups. To further analyze the relationships among Ct.Po, BV/TV, and BMD, correlation analysis was performed using GraphPad Prism 9.5 software, and the Pearson correlation coefficient was employed to quantify the strength of the relationships between the parameters. As shown in Fig. 5d, the Pearson correlation coefficient (r) between Ct.Po and BV/TV is 0.8459, which is significant at the 0.01 level, indicating a significant positive correlation; the Pearson correlation coefficient between Ct.Po and BMD is 0.7097, which is significant at the 0.05 level (Fig. 5e), demonstrating a significant positive relationship between the two.

By examining the scatter plots from the correlation analysis (Fig. 5d and e), two distinctly separated groups can be observed, each representing different states of bone preservation. The Fragile Group on the left side has lower values for Ct.Po, BV/TV, and BMD, indicating poorer preservation; while the Dense Group on the right side shows higher values for all three indicators, suggesting relatively better preservation.

These findings underscore the utility of Micro-CT technology in not only visualizing the microstructure of archaeological bone samples but also in quantifying their preservation status through the analysis of key microstructural parameters. The significant positive correlations identified between Ct.Po, BV/TV, and BMD provide valuable insights into the interplay of these parameters in the context of bone preservation. The clear distinction between the Fragile and Dense Groups highlights the impact of post-depositional processes on the integrity of the skeletal remains and offers a basis for further research into the factors influencing the long-term preservation of human bones in archaeological contexts.

The effect of age of death on bone preservation

The analysis results indicate that the preservation status of the nine samples can be primarily divided into two categories: the Dense Group, which is relatively well-preserved, and the Fragile Group, which is in a poorer state of preservation. Before delving into the discussion of the micro-preservation characteristics of the Dense and Fragile Groups, it is necessary to first eliminate biases related to the age at death.

The correlation between age and the state of skeletal preservation is complex and multifactorial. Age progression can precipitate physiological aging phenomena such as articular degeneration and osteoporosis, which are exacerbated with the passage of time. Additionally, lifestyle variations and activity levels across different age groups may also influence the preservation status of the skeletal remains.

To control for the confounding effects of age at death and pathology, Table 4 presents the sex, age, and preservation group for the nine samples under study. It is important to note that this study has excluded samples
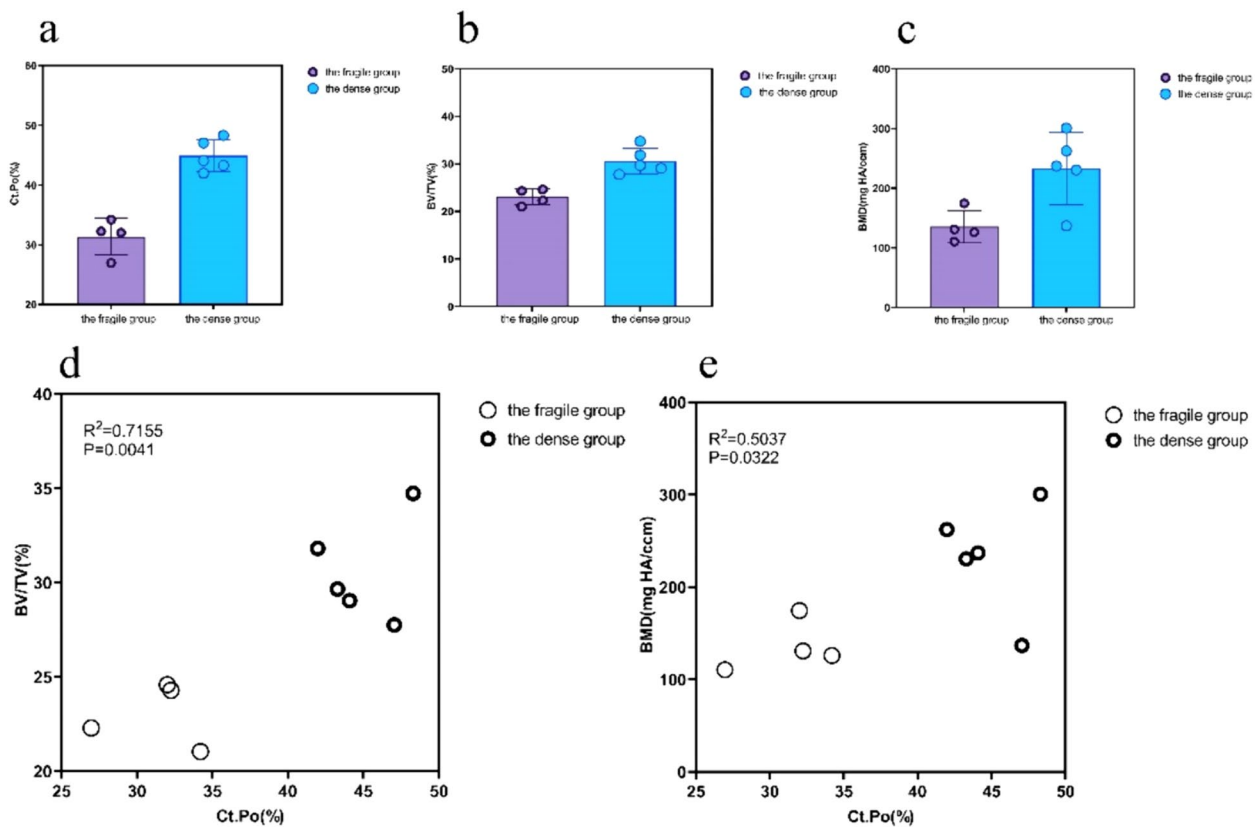
Fig. 5 Comparison of the difference in bone microstructure parameters between the fragile group and the dense group and scatterplot of correlation analysis of Ct. Po, BV/TV and BMD for nine samples. **a**, **b** and **c** show the visual results of the inter-group differences in Ct.Po, BV/TV and BMD between the fragile group and the dense group; **d** shows the correlation analysis between Ct.Po and BV/TV; **e** shows the correlation analysis between Ct.Po and BMD

Table 4 Distribution of sex, age of death and preservation group of 9 samples

Age in years	Age stage		Females(No.)	Males(No.)	Fragile group(No.)	Dense group(No.)
≤ 14	Non-adults		0	0	0	0
15–23	Adults	Young adults	2	0	2	0
24–35		Adults	2	3	1	4
36–55		Middle age	0	0	0	0
≥ 56		Senescence	1	1	1	1

showing signs of pathological changes to ensure that the observed micro-preservation characteristics are not affected by diseases that could influence bone density and tissue structure.

Upon examination of the nine samples in this study, the age at death is notably spread across the following life stages: young adults (15–23 years), adults (24–35 years), and senescence (60 years and above). The Fragile Group exhibits a presence in all these age brackets, with a pronounced representation in the young adults stage. In contrast, the Dense Group is exclusively found within the adults and senescence stages, with a marked predilection for adults. Moreover, Bello et al.[35] have posited that the preservation condition of skeletal remains increases proportionally with the individual's age, with the BMD in the skeletal remains of individuals aged 0–19 years being comparatively lower, and thus, not as well-conserved as that of adults. In the context of the two samples from the senescent stage, the observed bone substance loss could be attributable to age-related osteoporosis and

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degenerative joint diseases. The study's findings also indicate that the impact of age on bone preservation is not unidimensional but rather interlaces with other factors, including bone density and skeletal size [35, 36]. Bones from individuals in adults appear to be more consistently preserved relative to those from the young adults and senescent stages. It is also essential to account for variations in the burial environment, the depth of interment, and the funerary practices of different historical periods, which in turn affect the extent of archaeological human bone preservation.

However, with a sample size of only nine in this study, we must exercise caution in drawing definitive conclusions regarding the causal relationship between age and preservation status. Despite the limited sample size, our findings suggest that the categorization of preservation status in this study does not exhibit substantial variation across different age stages, thereby validating the reliability and efficacy of the Micro-CT analytical results. This study provides significant cues for a deeper comprehension of the micro-preservation characteristics of bones and effectively mitigates biases attributable to individual variability.

By focusing on samples from healthy individuals and correlating our Micro-CT derived groupings with the age at death, we have aimed to provide a comprehensive understanding of bone preservation that is not skewed by the age of the individual at the time of death. This approach has fortified the robustness of our conclusions, contributing to the overall reliability and effectiveness of our study.

Analysis of Micro-CT assessment of bone preservation

While Tripp et al. [26] suggested that Ct.Po is inversely proportional to the content of collagen in bones and that Ct.Po can serve as an indicator for assessing the preservation state of collagen, the Micro-CT scan results of this study show that the Fragile Group includes samples LDR490, LDR489, LDR522, and BJC486 (Fig. 6). This group has a lower Ct.Po values (25–35%) and exhibits characteristics of fragility and susceptibility to fracture in macroscopic descriptions (as detailed in Table 1).The average surface hardness values for these samples range from 4.67 to 5.33 (Table 1). Except for BJC486, all other samples in this group fractured during the sampling process. Micro-CT imaging also revealed that all but LDR522 from the Fragile Group showed porous features. However, the lower Ct.Po of the Fragile Group does not necessarily imply a higher preservation of collagen content, and relying solely on porosity to judge the preservation state of bones is a rather one-sided approach.

Although Micro-CT provides high-resolution imaging, it is not sufficient to observe pore sizes at the nanometer
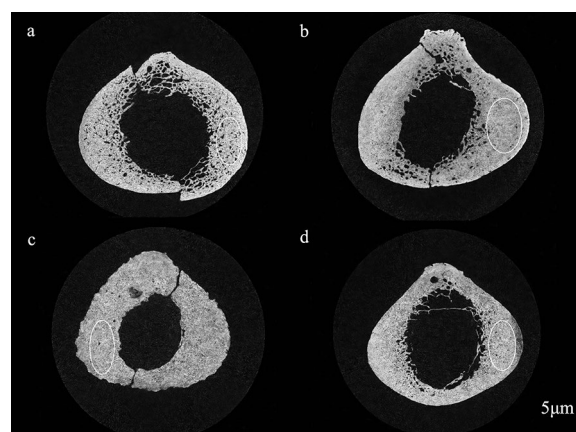

Fig. 6 Micro-CT image of the fragile group. From left to right, **a** LDR490; **b** LDR489; **c** LDR522; **d** BJC486

scale, and it cannot rule out the possibility that micropores ('s' porosity, diameter < 10 nm) in bones with low porosity and low collagen content may not be observable in Micro-CT but only pores larger than the spatial resolution limit can be detected, which may lead to a systematic underestimation of cortical porosity [37]. On the other hand, the increased porosity observed in the more poorly preserved samples is indicative of a greater extent of diagenesis, which can compromise the structural integrity of the bone and complicate the interpretation of pathological conditions or trauma. The increased porosity could be a result of several factors, including the loss of organic matrix, such as collagen, and the subsequent mineralization of the bone tissue. This process can lead to a more fragile bone structure, with a higher risk of fragmentation and erosion over time. What can be confirmed is that the low BMD and BV/TV indicators in the Fragile Group reflect the loss of minerals and bone matrix in the bone and a weakening of bone strength.

The Dense Group includes samples LDR521, LDR520, BJC487, SXP491, and SXP488 (Fig. 7). This group has a higher Ct.Po of 40–50%, and the higher BMD and BV/TV also indicate that the bone quality of this group is superior to that of the Fragile Group. Macroscopic descriptions (refer to Table 1) depict most samples in the Dense Group as having a more robust bone quality. This is further supported by the average surface hardness values, which fall between 5.83 and 6.83. Micro-CT imaging of the Dense Group reveals fewer pores and notable macropores, with a higher brightness in the cortical bone, indicating a dense and less fragile bone structure that is less prone to fracture. These Micro-CT findings are consistent with the macroscopic examination results, confirming the integrity and compactness of the bone in the Dense Group.
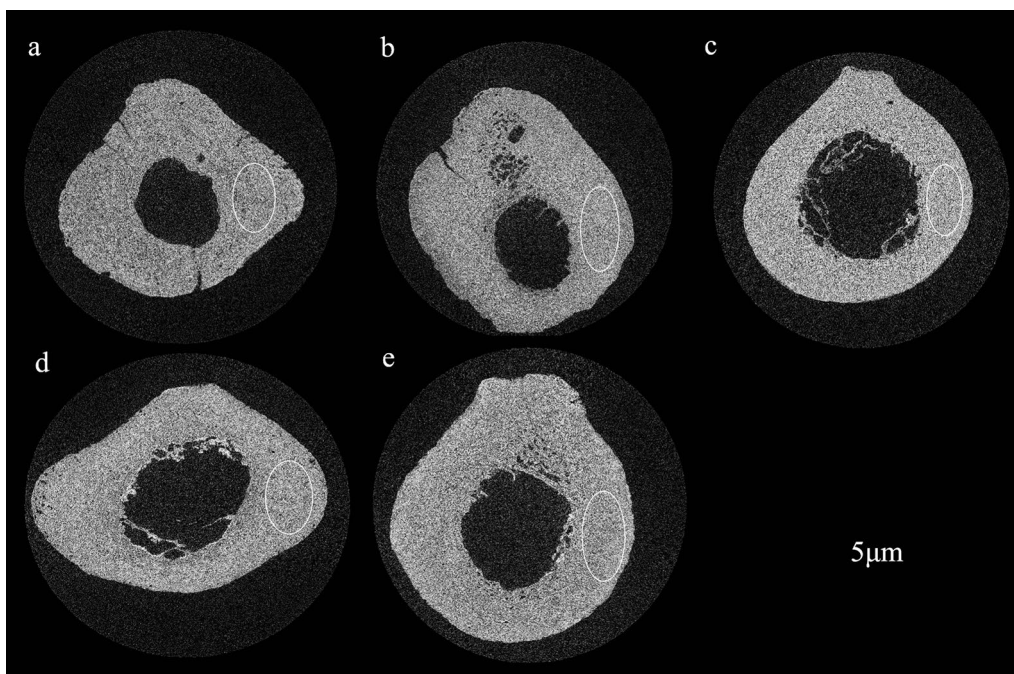
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Fig. 7 Micro-CT image of the dense group. From left to right, **a** LDR521; **b** LDR520; **c** BJC487; **d** SXP491; **e** SXP488

These findings highlight the complexity of bone preservation in archaeological contexts and the importance of using a combination of imaging techniques and microstructural parameter analysis to accurately assess the state of preservation. The distinction between the Dense and Fragile Groups not only aids in understanding the post-depositional changes that bones have undergone but also informs strategies for the conservation and study of archaeological bone samples.

Limitations of the Micro-CT in assessing archaeological bone

In the field of archaeological research, Micro-CT technology has demonstrated its potential in studying the diagenetic processes and biological erosion of bones [24]. Despite its advantages, Micro-CT technology also has certain limitations. Like other X-ray analysis techniques, Micro-CT struggles to differentiate between minerals of biological origin and other mineral impurities [38]. Additionally, the imaging results obtained from Micro-CT can be affected by the actual condition of the samples and the differences in pre-treatment methods.

It is important to recognize that Micro-CT remains a powerful tool for multi-faceted understanding of the preservation status of archaeologically excavated human bones due to its high-resolution at the micrometer scale, non-destructive scanning capabilities for small samples, and rapid acquisition of parameter information. The

integration of Micro-CT technology with archaeological and anthropological research highlights the importance of interdisciplinary approaches in advancing our understanding of human history and biology.

In summary, while Micro-CT technology has its limitations, its strengths in high-resolution imaging and non-destructive analysis make it an invaluable tool in the study of archaeological bone preservation. It complements other analytical techniques and contributes to a more comprehensive understanding of the complex processes that affect the long-term preservation of human remains in archaeological contexts. It should be noted that although our study provides a preliminary assessment framework for the micro-preservation status of archaeologically unearthed human bones using Micro-CT technology, it does not encompass a detailed exploration into pathogen presence, protein preservation, radiocarbon dating, anthropological age estimation, or paleoanthropological disease analysis. These aspects are significant for a comprehensive understanding of the bones' archaeological context and will be the focus of our future research endeavors.

Conclusion

In this study, we employed Micro-CT technology to attempt an assessment of the micro-preservation status of nine ancient human femur samples unearthed from three different archaeological sites. It proposes Micro-CT

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scanning parameters and microstructural parameters suitable for the preservation status of archaeologically excavated human bones. By integrating macroscopic observations, Micro-CT imaging results, and the calculated results of three parameters—Ct.Po, BV/TV, and BMD—we have preliminarily identified two distinct preservation types. This classification provides a reference for the establishment of preservation grade standards for archaeologically excavated human bones. The study concludes the following regarding the micro-preservation status of archaeological bones:

1. Micro-CT as a Valuable Tool: The concordance between the Micro-CT observations and the results of macroscopic examination, along with average surface hardness measurements, underscores the effectiveness of Micro-CT as a tool for assessing the micro-preservation status of archaeological human bones. It also confirms its complementary role to traditional macroscopic descriptive assessments commonly used in archaeological fieldwork. This method ensures that assessments are not entirely reliant on a subjective interpretation of the bone's preservation condition but are instead supported by empirical data from both macroscopic and microstructural analyses.

2. Key Microstructural Parameters: Cortical porosity (Ct.Po), bone volume fraction (BV/TV), and bone mineral density (BMD) serve as critical indicators for assessing the micro-preservation status of archaeologically excavated human bones. Micro-CT technology offers a novel method for analyzing these microstructural characteristics.

3. Identification of Bone Preservation Groups: The preliminary identification of two preservation types—Dense Group and Fragile Group—based on the microstructural parameters, allows for a more nuanced understanding of the factors affecting bone preservation. Samples with lower cortical porosity (25–35%), bone volume fraction (20–25%), and bone mineral density values, which macroscopically exhibit fragile, porous, and spongy characteristics, indicate poor preservation. In contrast, samples with relatively higher cortical porosity (40–50%), bone volume fraction (27–35%), and bone mineral density values, which macroscopically appear solid and dense, indicate better preservation. This classification can be used to guide bone conservation efforts.

4. Feasibility and Effectiveness of Micro-CT: In the realm of studying the micro-preservation status of archaeologically unearthed human bones, the consistency between the results of Micro-CT and the macroscopic preservation conditions in this study demonstrates the feasibility and effectiveness of the technology. Through the detailed analysis provided by Micro-CT, it is possible to identify micro-fractures, porosity, and other indicators of preservation quality that are not visible to the naked eye. This lays the groundwork for future research that integrates multidisciplinary methods, such as mechanical properties and burial environment indicators, to establish an on-site comprehensive assessment model for the preservation status of archaeologically excavated human bones. The use of Micro-CT, therefore, enhances the objectivity and precision of osteological preservation assessments in archaeology, which is particularly beneficial for determining the suitability of bones for further scientific analyses such as isotopic analysis. Moreover, the consistency of results across different methods instills confidence in the application of Micro-CT as a primary or auxiliary tool in archaeological research.

These conclusions underscore the importance of Micro-CT technology in the field of archaeological research, particularly in the study of human skeletal remains. Also, the alignment of Micro-CT scan outcomes with macroscopic observations and hardness measurements serves to validate the preservation status groupings made in this study. This alignment also supports the broader application of Micro-CT in archaeology, augmenting the analytical tools available for the study and conservation of archaeological human bones.

In summary, the application of Micro-CT in the study of archaeological human bones has proven to be a valuable tool for assessing micro-preservation status. It provides a level of detail that complements traditional macroscopic analyses, allowing for a more thorough and accurate understanding of the preservation conditions of ancient skeletal remains. The findings contribute to the development of standardized methods for analyzing and preserving archaeological human remains. By establishing standardized scanning protocols and analyzing microstructural parameters, researchers can gain a deeper understanding of the complex processes that influence the preservation of human remains, which is essential for the interpretation of our biological and cultural heritage. While our study provides preliminary insights, it also acknowledges the limitations imposed by the sample size and the need for further research with a broader range of samples and contexts to validate and expand upon these initial findings.

the main manuscript. YX and XL revised the main manuscript. All authors read and approved the final manuscript.

Data availability
In this work, the original data are shown in the main manuscript, and any other further data are available upon request from the authors.

Declarations

Ethic approval and consent to participate
This article does not contain any studies with living human participants performed by any of the authors. All experimental samples were obtained from archaeological excavations. Ethics Committee approval was obtained from the Institutional Ethics Committee of School of Culture Heritage, Northwest University to the commencement of the study. Moreover, we followed guidance for the ethical treatment of human remains following "Ethics of DNA research on human remains: five globally applicable guidelines" (https://www.nature.com/articles/s41586-021-04008-x).

Consent for publication
This article does not contain any studies with human participants performed by any of the authors. All information reported in this paper is anonymized and the submission does not include images that may identify the person.

Competing interests
The authors declare that they have no competing interests. We declare that we do not have any commercial or associative interest that represents a competing interest in connection with the work submitted.

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