ULTRAFILTRATION PRETREATMENT FOR ^{14}C DATING OF FOSSIL BONES FROM ARCHAEOLOGICAL SITES IN JAPAN

M Minami[1,2] • K Sakata[3] • M Takigami[4] • T Nagaoka[5] • T Nakamura[1]

ABSTRACT. To study the effect of ultrafiltration on the radiocarbon ages of relatively poorly preserved bones in Japan, we analyzed the ^{14}C dates of high-molecular-weight (HMW) gelatin samples and compared them with those of other extracted organic fractions, unfiltered gelatin samples extracted from NaOH-treated or NaOH-untreated collagen, and XAD-purified hydrolysates of animal fossil bones (~4600 BP; gelatin yield of 2–4%) from the Awazu underwater archaeological site, Shiga, Japan. NaOH-treated, unfiltered gelatins and XAD-purified hydrolysates showed statistically similar ^{14}C ages to those of HMW gelatins. The ^{14}C ages of the HMW gelatins were the oldest and similar to those of wood collected from the same layer as the bones, and the NaOH-treated, unfiltered gelatins gave ^{14}C ages within the acceptable margins of error; therefore, ultrafiltration was effective for accurate ^{14}C dating, while NaOH-treated gelatin without ultrafiltration was also sufficient to obtain accurate ^{14}C dates on the animal bones. The ^{14}C ages of human skeletons (~750 BP; gelatin yield of 2–11%) from 5 individuals excavated from an archaeological site in Yuigahama, Kamakura, Japan, showed statistically the same ^{14}C ages as NaOH-treated, unfiltered gelatins and HMW gelatins within the margins of error, although HMW gelatins were likely to give slightly older ages than unfiltered gelatin with a yield of less than ~3%. These results indicate that unfiltered gelatins extracted from fossil bones of gelatin yield more than ~3% can produce accurate ^{14}C ages without the need for ultrafiltration. Ten bone fragments from 3 humans showed the same ^{14}C ages for each individual, suggesting that any bone part from an individual can be used to obtain a representative age. The ^{14}C ages of tooth enamels of 2 individuals were 35 and 70 yr older than their bone ages. Death dates obtained from these age gaps agreed with those determined by morphology.

INTROUCTION

Bone collagen is commonly used for radiocarbon dating in many laboratories, but bone collagen often suffers from diagenesis and can be contaminated with foreign materials, such as humic and fulvic acids, yielding erroneous ^{14}C ages. For more accurate ^{14}C dating, gelatin extraction is commonly used to remove adsorbed organic matter from bone protein (Longin 1971), but it is likely that gelatin extraction alone does not fully remove contaminants. NaOH treatment of decalcified bone is effective in removing contaminants to get accurate ^{14}C ages (Arslanov and Svezhentsev 1993; Minami et al. 2004), although this treatment can lead to considerable loss of organic bone protein. Bones can be also accurately dated bones by pretreating them with the absorbent polymeric resin XAD-2 (e.g. Stafford et al. 1988). This process is effective for removing foreign materials such as humates and humic acids without the loss of organic bone protein, but it requires considerable labor, particularly with regard to pre-cleaning the XAD-2 resin (Minami et al. 2000). The recent trend is to use ultrafiltration as a post-preparation technique for bone gelatin. This method was originally proposed by Brown et al. (1988) as a protocol to be used in addition to Longin's (1971) collagen extraction. Ultrafiltration removes low-molecular-weight (LMW) contaminants at 30kDa MW cut-offs as well as degraded proteins while preserving select high-molecular-weight (HMW) and intrinsic proteins, which are more likely to be derived from the original collagen present in the bone (Bronk Ramsey et al. 2004). Therefore, ultrafiltration can be used to obtain more accurate ^{14}C dates of bones. However, Minami et al. (2013b) reported that the ^{14}C ages, as well as the δ^{13}C and δ^{15}N values of unfiltered gelatins, were in good agreement with the values of HMW gelatins for some well-preserved VIRI bone samples, suggesting that unfiltered gelatins extracted from well-preserved

[1]Center for Chronological Research, Nagoya University, Nagoya 464-8602, Japan.
[2]Corresponding author. Email: minami@nendai.nagoya-u.ac.jp.
[3]Graduate School of Environmental Studies, Nagoya University, Nagoya 464-8601, Japan.
[4]Faculty of Literature and Social Sciences, Yamagata University, Yamagata 990-8560, Japan.
[5]Department of Anatomy, St. Marianna University School of Medicine, Kanagawa 216-8511, Japan.

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bones could be used to obtain accurate ^{14}C dates without ultrafiltration. Furthermore, ultrafiltration might not always yield correct ^{14}C dates, because the ultrafiltration step could introduce contaminants to a sample, mainly via the type of filters used and an inadequate precleaning regimen (Bronk Ramsey 2004; Brock et al. 2007; Hüls et al. 2007, 2009; Minami et al. 2012, 2013b).

To study the effects of ultrafiltration on ^{14}C ages of relatively poorly preserved bones, we compared the ^{14}C ages of HMW gelatins with those of unfiltered gelatins extracted from NaOH-treated or untreated collagens, and with samples of XAD-purified hydrolysates of animal fossil bones excavated at the Awazu submerged archaeological site of Lake Biwa, Shiga, Japan. We also measured the ^{14}C ages of unfiltered and HMW gelatins from different parts of human skeletons excavated from a Medieval archaeological site in the Yuigahama area, Kamakura, Japan, to determine whether every bone part in an individual shows the same ^{14}C age for unfiltered and HMW gelatins, and to determine whether a measured age from a certain part of a human skeleton is representative of the age of that individual.

EXPERIMENTAL METHODS

Samples

The animal bone fragments were collected at a shellmound excavated at the Awazu underwater archaeological site. Samples AWA-8 to -14 are bone fragments from deer (*Cervus nippon*) and boar (*Sus scrofa*). The bone samples were previously dated on gelatins extracted from decalcified bone collagens without NaOH treatment (Nakamura et al. 1997) and XAD-treated gelatin hydrolysates (Minami and Nakamura 2000). Wood samples collected from the same layers were also previously dated (Nakamura et al. 1997). In this study, ^{14}C ages of HMW gelatins and unfiltered gelatins extracted from NaOH-treated collagens were measured.

Human skeletal samples were collected from 5 individuals (YM121A, YM5001, YM5654, YM18B-1, and YM18B-2) excavated at a Medieval archaeological site in the Yuigahama area, Kamakura. YM121A is a female (45–54 yr old), YM5001 is a male (35–44 yr old), and YM5654 is a female (45–54 yr old) (Hirata et al. 2002). Eleven skeletal parts (tooth dentin, cranium, sphenoid, rib, dorsal vertebra, lumbar vertebra, humerus, radius, central part of femur, lower part of femur, and tibia) from each individual of YM121A, YM5001, and YM5654 were analyzed. YM18B-1 and YM18B-2, which were buried together, are female and male individuals, respectively, 25–34 yr old, and were expected to yield the same ^{14}C ages. For sample YM5654, a rib had already been used to measure ^{14}C ages of 739 ± 30 BP (NUTA2-10200) and 722 ± 31 BP (NUTA2-10213) (Minami et al. 2013b). Additional unfiltered gelatins of another rib of YM18B-1 and YM18B-2 had also been measured and yielded values of 819 ± 39 BP (NUTA2-12414) and 758 ± 40 BP (NUTA2-12415), respectively (Minami et al. 2013b).

Gelatin Extraction from Skeletal Samples

Extraction of collagen from bone and a tooth was performed using our standard preparation procedure (Minami and Nakamura 2000, 2005; Minami et al. 2004). Each sample was repeatedly ultrasonicated in Milli-Q™ water and then in 0.2M NaOH to remove surface contaminants. After being rinsed with Milli-Q water, the sample was lyophilized and pulverized. The resulting bone and tooth powders were decalcified with 0.6M HCl in a precleaned cellulose tube membrane (Viskase Sales Corp.) with a molecular cut-off of 12,000–14,000 in a beaker at 4 °C for 24 hr. After dialysis with Milli-Q water, the contents of the cellulose tube were centrifuged and lyophilized. The decalcified sample was then treated with 0.1M NaOH at room temperature. The NaOH-treated

collagen was washed with Milli-Q water repeatedly, then treated with 0.6M HCl overnight at 4 °C to remove atmospheric carbon added during the NaOH treatment. The collagen was rinsed with Milli-Q water. Gelatin was extracted from the acid/alkali-insoluble residue by heating the fraction in slightly acidic (pH ~3) water at 90 °C for 12 hr.

Ultrafiltration

Vivaspin™ 6 (VS 6, a polyethersulfone membrane, PES) ultrafilters with a 30,000 molecular weight cut-off (MWCO), were cleaned by the method described by Bronk Ramsey et al. (2004). The filters were rinsed twice by centrifugation with Milli-Q water, ultrasonicated in Milli-Q water, and then rinsed again by centrifugation. About 5060 mg of gelatin dissolved in ~5 mL of Milli-Q water was put on the filter and centrifuged at 3000 rpm for ~2.5 hr until ~1 mL of HMW gelatin and 4 mL of LMW fraction remained. Prolonged ultrafiltration of gelatin may introduce contaminants (Minami et al. 2013a); therefore, we limited ultrafiltration time to less than 2.5 hr. The HMW gelatin and LMW fractions obtained from the centrifugation were lyophilized separately.

Sample Combustion and ^{14}C Analysis

Each fraction of unfiltered gelatin and HMW gelatin was vacuum-sealed in a quartz tube with CuO, Cu, and Ag and combusted at 850 °C for 4 hr. The CO_2 gas produced from the reaction was purified cryogenically and then reduced to graphite by H_2 with an Fe catalyst at 620 °C for 8 hr in a sealed quartz tube. The samples (<0.5 mg C) were graphitized with the compact graphitization system for small-sized samples described in Minami et al. (2013a). The graphite was ^{14}C dated by the Tandetron accelerator mass spectrometer (HVEE model 4130-AMS) at Nagoya University, Japan. For ^{14}C dating, NIST HOx-II served as the ^{14}C standard, and Kishida® oxalic acid containing ^{14}C-free carbon served as the ^{14}C background sample.

RESULTS AND DISCUSSION

Animal Bone Fragments from the Awazu Archaeological Site

The results in Table 1 are from 3 fractions of sequential chemical purification of samples AWA-10 to -14. ^{14}C ages for the sequential chemical extractions of the animal bone samples AWA-8 to -14 are plotted in Figure 1, together with the results from Nakamura et al. (1997) and Minami and Nakamura (2000).

Table 1 Analyses of animal bone gelatins from the Awazu archaeological site.

Fraction	Gelatin yield[a] (%)	$\delta^{13}C$[b] (‰)	^{14}C age[c] (BP)	Lab code (NUTA2-)
AWA-10	**2.2**			
NaOH-treated, unfiltered gelatin		–22.0	4540 ± 26	18432
HMW gelatin		–20.0	4601 ± 79[d]	18631
LMW fraction		–22.2	4543 ± 25	18435
AWA-11	**1.9**			
NaOH-treated, unfiltered gelatin		–22.5	4507 ± 25	18438
HMW gelatin		–23.0	4575 ± 27	18433
LMW fraction		–24.4	4362 ± 28	18436
AWA-12	**3.8**			
NaOH-treated, unfiltered gelatin		–24.0	4606 ± 28	18434
HMW gelatin		–20.7	4667 ± 60[d]	18635
LMW fraction		–24.9	4567 ± 26	18437

Table 1 Analyses of animal bone gelatins from the Awazu archaeological site. *(Continued)*

Fraction	Gelatin yield[a] (%)	$\delta^{13}C$[b] (‰)	^{14}C age[c] (BP)	Lab code (NUTA2-)
AWA-13	**2.1**			
NaOH-treated, unfiltered gelatin		–22.4	4495 ± 25	18441
HMW gelatin		–22.1	4561 ± 26	18443
LMW fraction		–22.2	4425 ± 25	18442
AWA-14	**2.4**			
NaOH-treated, unfiltered gelatin		–21.9	4521 ± 25	18440
HMW gelatin		–22.4	4603 ± 58[d]	18636
LMW fraction		–22.4	4668 ± 26	18444

[a]Weight percentages of NaOH-untreated, unfiltered gelatins relative to the starting bone weight.
[b]Measured by AMS. The values have an error of ~1‰.
[c]Measurement error associated with ^{14}C dating is 1σ.
[d]Values were obtained by use of the compact graphitization system.

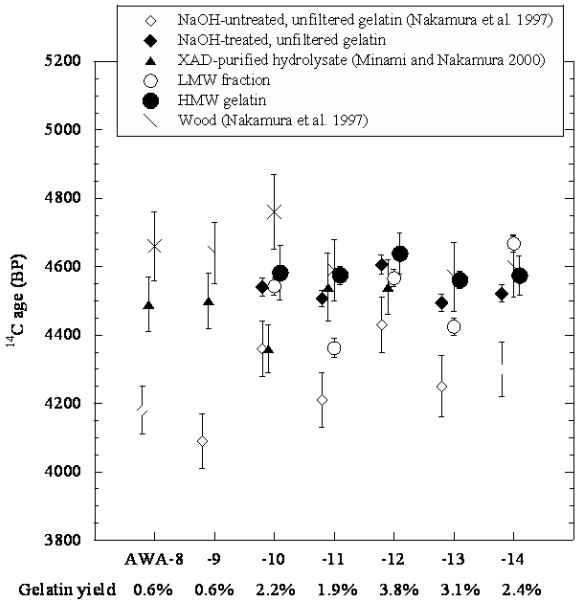

Figure 1 ^{14}C ages of organic fractions from animal fossil bones and wood, Awazu submerged archaeological site, Shiga, Japan. The data on NaOH-untreated, unfiltered gelatins and wood are from Nakamura et al. (1997), and the data on XAD-purified gelatin hydrolysates are from Minami and Nakamura (2000).

The 5 organic fractions in AWA-10 to -14 gave the oldest ages on HMW gelatins, and were statistically indistinguishable ($p = 0.32 > 0.05$) from the dates for the wood that was collected from the same layer as the bones (Figure 1). Minami and Nakamura (2000) reported that the ^{14}C age of fulvic acids intruding into the animal bones used in this study were significantly younger (~3900 BP) than those of the bone organic carbon. Therefore, bone gelatins can give younger ages if exogenous organic contaminants are not totally removed. LMW fractions of AWA-11 and -13 tended to have younger ^{14}C ages than HMW gelatins and wood samples, indicating the presence of exogenous carbon. HMW gelatins could therefore give more accurate ages.

The XAD-purified hydrolysates gave the same ages as the NaOH-treated, unfiltered gelatins, which were similar to those of the HMW gelatins within measured errors except for sample AWA-10. Thus, purification with XAD-2 resin for the NaOH-treated, unfiltered gelatins appeared to be not that effective in this case, probably due to the previous removal of exogenous carbon (which a post-XAD purification can remove) originating from burial environments. Taking into consideration that foreign organic carbon from XAD resin might contaminate bone fractions and lead to erroneous ^{14}C ages when XAD resin is not sufficiently precleaned, gelatin extraction from NaOH-treated collagens without XAD purification appears to be satisfactory to obtain accurate ^{14}C ages.

The ^{14}C ages of unfiltered gelatins extracted from NaOH-untreated collagens were about 200 yr younger than those of the other fractions including NaOH-treated, unfiltered gelatins. This result suggests the presence of organic contaminants in the NaOH-untreated, unfiltered gelatins, which were removed from the NaOH-treated, unfiltered gelatins. During diagenesis, bone protein is degraded by breakdown of collagen fibrals, and it becomes increasingly difficult to separate bone protein from foreign organic matter. Hot-water extraction is unlikely to fully remove exogenous organic matter from poorly preserved fossil bones containing <1% extractable gelatin (Minami and Nakamura 2000). The bone samples used in this study had acceptable gelatin yields of 1.9–3.8% and the NaOH-untreated, unfiltered gelatins showed younger ages. Therefore, our experiments suggest that NaOH treatment of collagen before gelatin extraction is necessary for bone samples to remove foreign contaminants sufficiently to obtain accurate ^{14}C ages, consistent with the results of Arslanov and Svezhentsev (1993) and Minami et al. (2004).

The membrane tube with a molecular cut-off of 12,000–14,000 used during sample decalcification with HCl also helped to filter most of the LMW contaminants and degraded proteins while simultaneously retaining HMW collagens. The fact that we obtained statistically the same ages ($p = 0.0001$) for the NaOH-treated, unfiltered gelatins and the HMW gelatins could be due to removal of LMW contaminants on decalcification. We propose that NaOH treatment of bone collagen decalcified in a membrane tube prior to gelatin extraction can improve resultant ^{14}C ages, and this decalcification method is also easy to use.

Human Bones in the Yuigahama Archaeological Site

Table 2 shows the C/N ratios, gelatin yields, and ^{14}C ages of the human bone and tooth samples. The C/N ratios of the bone and tooth powders prior to chemical treatment were 5.2–7.1 (average 5.9), 3.7–5.1 (average 4.4), and 3.8–4.4 (average 4.2) for YM121A, YM5001, and YM5654, respectively, whereas the C/N ratios of the gelatins extracted from the NaOH-treated collagens (without MW filtration) were 3.1–3.4 for all samples, which is within the acceptable range of 2.9–3.5 for collagen (van Klinken 1999). This result suggests that foreign organic contaminants in the bone could be effectively removed during gelatin extraction, which incorporates the NaOH step following decalcification of bone powders. The yields of gelatins were 1.6–4.4 wt% (average 3.0), 4.7–7.8 wt% (average 6.3), and 5.6–11.0 wt% (average 7.9) for YM121A, YM5001, and YM5654, respectively. A positive relationship was observed between C/N ratios of skeletal powders and yields of extracted gelatins (Figure 2): The YM121A skeletal powders with a higher average C/N ratio of 5.9 had gelatin yields averaging only 3.0 wt%, and the YM5654 skeletal powders with a lower average C/N ratio of 4.2 showed gelatin yields averaging 7.9 wt%. These results suggest that YM121A was less well preserved than YM5001 and YM5654, and that the NaOH-treated, unfiltered gelatins of YM121A were likely to give inaccurate ^{14}C ages.

The ^{14}C ages for YM121A gelatins can be combined by the R_Combine function of the OxCal v 4.2 program (Bronk Ramsey 2009), resulting in 710 ± 7 and 738 ± 13 BP for unfiltered gelatins and

Table 2 Analyses of human bone and tooth collagens from the Yuigahama archaeological site.

	Bone (tooth) powder	NaOH-treated, unfiltered gelatin				HMW gelatin	
	C/N[a]	C/N[a]	Yield[b] (wt%)	^{14}C age[c] (BP)	Lab code (NUTA2-)	^{14}C age[c] (BP)	Lab code (NUTA2-)
YM121A							
Tooth (third molar)	5.0	3.2	4.6	784 ± 23	18209	770 ± 31	17746
Cranium	6.0	3.3	3.1	686 ± 22	11494	747 ± 25	18215
Sphenoid	5.2	3.3	5.7	733 ± 22	11482	736 ± 25	17745
Rib	5.5	3.3	3.8	716 ± 22	11484	702 ± 50[d]	18622
Dorsal vertebra	5.9	3.3	4.4	734 ± 22	11492	750 ± 57[d]	18627
Lumbar vertebra	7.1	3.1	1.6	685 ± 22	11493	722 ± 62[d]	18628
Humerus	5.9	3.3	2.2	700 ± 22	11486	—	—
Radius or ulna	5.3	3.4	1.9	716 ± 22	11487	—	—
Central part of femur	5.7	3.3	2.5	691 ± 22	11477	741 ± 26	17747
Lower part of femur	5.8	3.3	2.8	710 ± 22	11485	744 ± 58[d]	18626
Tibia	7.0	3.4	2.2	731 ± 22	11491	—	—
YM5001							
Tooth (canine tooth)	4.5	3.2	5.7	775 ± 28	12888	764 ± 25	17732
Rib	4.7	3.2	6.1	697 ± 28	12889	664 ± 22	18216
Dorsal vertebra	—	3.2	4.7	692 ± 28	12896	704 ± 25	17740
Lumbar vertebra	—	3.2	7.8	750 ± 29	12897	700 ± 25	17744
Humerus	5.1	3.2	4.7	684 ± 28	12891	702 ± 25	17736
Radius or ulna	4.3	3.2	8.8	675 ± 28	12892	669 ± 25	17737
Central part of femur	3.8	3.2	6.3	723 ± 28	12893	707 ± 25	17738
Lower part of femur	5.0	3.2	5.5	671 ± 28	12890	667 ± 25	17735
Tibia	3.7	3.2	6.1	675 ± 28	12895	720 ± 25	17739
YM5654							
Sphenoid	4.1	3.2	6.3	710 ± 25	17720	755 ± 24	18213
Rib	3.8	3.2	7.1	723 ± 25	17721	740 ± 23	18214
Dorsal vertebra	4.0	3.2	7.0	738 ± 25	17726	675 ± 25	17730
Lumbar vertebra	4.4	3.2	5.6	754 ± 25	17727	736 ± 25	17731
Central part of femur	4.2	3.2	11.0	793 ± 25	18217	793 ± 25	17729
Lower part of femur	4.4	3.2	10.6	797 ± 25	17722	768 ± 25	17728
YM18B-1							
Rib	4.9	3.3	6.7	785 ± 45	14638	743 ± 45	14641
(duplicated)						765 ± 45	14642
YM18B-2							
Rib	4.5	3.3	6.3	715 ± 45	14643	776 ± 45	14644
(duplicated)						718 ± 45	14645

[a]Atomic C/N ratio.
[b]Weight percentages are reported relative to the starting bone weight.
[c]Measurement error associated with ^{14}C dating is 1σ.
[d]Values were obtained by using the compact graphitization system.

HMW gelatins, respectively. The ^{14}C ages for YM5001 and YM5654 can be also combined to 696 ± 10 and 692 ± 9 BP and 753 ± 11 and 745 ± 10 BP (except the dorsal vertebra part) for unfiltered gelatins and HMW gelatins, respectively. Only HMW gelatin of the YM5654 dorsal vertebra cannot be combined due to the significantly younger age than the others, suggesting the presence of foreign contaminants. Ultrafiltration as a post-preparation technique can improve the accuracy of ^{14}C dating of bone gelatin, but it can also introduce contaminants. In the discussion to follow, the ^{14}C age of the HMW gelatin of YM5654 dorsal vertebra is thus not taken into consideration.

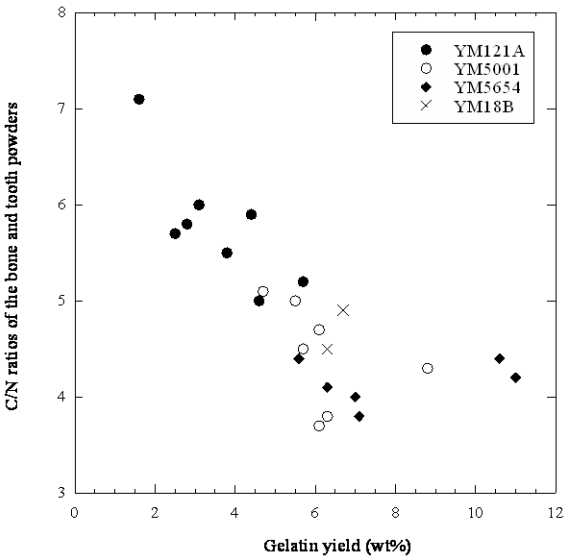

Figure 2 Relationship between gelatin yields and C/N ratios of bone and tooth powders of NaOH-treated, unfiltered gelatins from human individuals from a Medieval archaeological site in the Yuigahama area, Kamakura, Japan.

The unfiltered gelatins and HMW gelatins from YM5001 and YM5654 exhibited statistically the same ^{14}C ages ($p = 0.61$ and 0.62, respectively), whereas those from YM121A were likely to be a little different ($p = 0.07$) because some of the HMW gelatins from YM121A tended to give ages that were ~50 yr older than those of the unfiltered gelatins, whose yields were less than ~3% (Figure 3). This finding suggests that unfiltered gelatins with low yields could remain slightly contaminated, and that ultrafiltration might effectively remove such contaminants to provide more accurate ages for fossil bones with a preservation state similar to that of YM121A. However, most differences between the ages of unfiltered gelatins and HMW gelatins are small, within analytical errors, and so ^{14}C ages of unfiltered gelatins with gelatin yield more than ~3% can be acceptable.

Age Deviation of Different Skeletal Parts in Individuals

The ^{14}C ages of tooth dentins for YM121A (third molar) and YM5001 (canine tooth) were 35 and 70 yr older than the bones of these individuals, respectively. This age gap is due to the different metabolic systems of tooth and bone. Tooth formation stops at 4–5 yr of age on canine teeth and about 11–13 yr of age on third molars. Therefore, the ages of YM121A and YM5001 at death could be estimated from the ^{14}C values of their teeth and bones to be around 50 and 75 yr, respectively. These ages roughly agree with the ages estimated by use of the morphological method for YM121A (4554 yr), but not for YM5001 (3544 yr). The estimated ages from the ^{14}C analysis in this study have large errors; therefore, more detailed analyses of the teeth (e.g. analysis of divided fractions in the teeth) are necessary. This method could be useful for age determination of individuals because the traditional morphological method can be imprecise, especially in adults.

The ^{14}C ages of the different bone parts (excluding tooth) from the same individual were statistically similar within errors, and ^{14}C calibrated ages for YM121A, YM5001, and YM5654 (except HMW gelatin of dorsal vertebra part) can be combined by the R_Combine function of OxCal v 4.2 (Bronk Ramsey 2009), resulting in cal AD 1271–1288, 1276–1294, and 1257–1280 (95.4%), respectively.

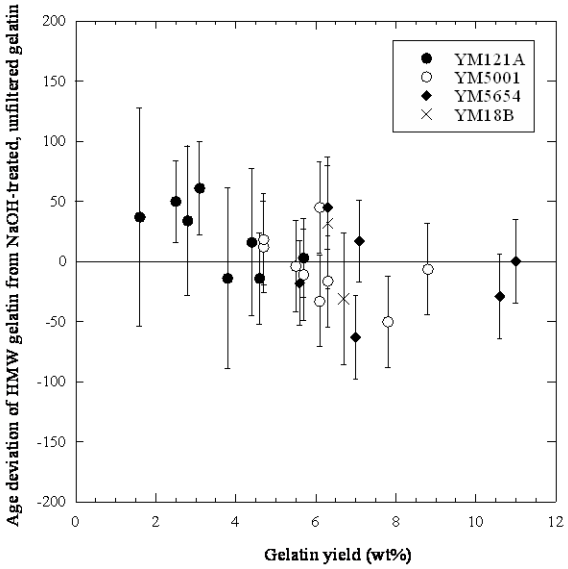

Figure 3 Relationship between gelatin yields and age deviation of HMW gelatin from NaOH-treated, unfiltered gelatin of NaOH-treated, unfiltered gelatins from human individuals from a Medieval archaeological site in the Yuigahama area, Kamakura, Japan.

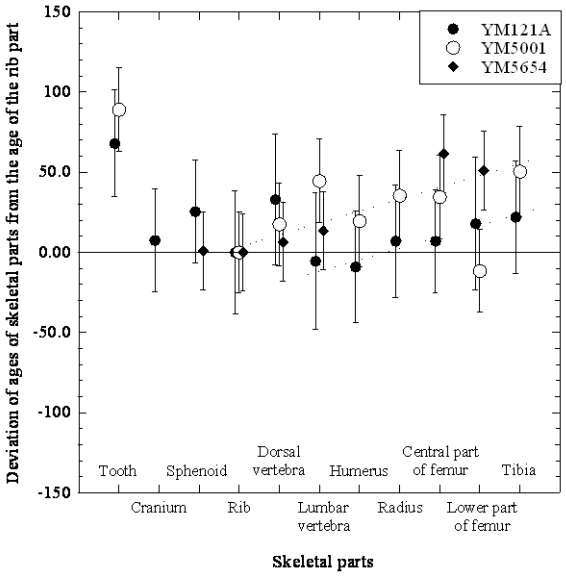

Figure 4 Deviation of ages of skeletal parts from the age of the rib part. Dotted lines show trends of the plots of YM121A and YM5001.

We can thus obtain a representative age by using any skeletal part for ^{14}C dating. The rib is likely to show the youngest ^{14}C ages within the margin of error, yet are still accurate. Figure 4 shows the age deviation of 11 skeletal parts relative to that of the rib for YM121A, YM5001, and YM5654 individuals. The average ages of unfiltered and HMW gelatins were used for calculation. These values

tended to increase from the dorsal vertebra, near the heart, to the tibia, which is far from the heart. This trend may be due to differences in metabolic rate. Future studies should address whether different ^{14}C ages are preserved in bone parts, by using data on carbon and nitrogen stable isotope ratios and amino acid composition.

CONCLUSIONS

We investigated the effect of ultrafiltration on the ^{14}C age of relatively poorly preserved bones, namely, animal fossil bones (~4600 BP; gelatin yield of 2–4%) collected from the Awazu underwater archaeological site, Shiga, Japan, and human skeletons (~750 BP; gelatin yield of 2–11%) of 5 individuals excavated from a Medieval archaeological site in the Yuigahama area, Kamakura, Japan. Our findings are as follows:

1. Ultrafiltration as a post-preparation technique was very effective for accurate ^{14}C dating of fossil bones analyzed in this study, whereas the gelatin extraction method with NaOH treatment was sufficient to obtain accurate ^{14}C ages within the margin of error for fossil bones of gelatin yield more than ~3%.
2. ^{14}C ages of the NaOH-untreated, unfiltered gelatins were substantially younger than those of the other fractions, whereas those of the NaOH-treated, unfiltered gelatins were the same as the ^{14}C ages of HMW gelatins, suggesting that the NaOH treatment of collagen before gelatin extraction effectively removed contaminants from the bones to allow for accurate ^{14}C age determination.
3. XAD-purified hydrolysates showed the same ^{14}C ages as those of the NaOH-treated, unfiltered gelatins; XAD purification was not as effective on the animal bones in this study.
4. ^{14}C ages of the different bone parts in an individual were the same (within the margin of error), indicating that a representative age can be obtained by using any skeletal part for ^{14}C dating.

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