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TESTING THE USE OF XAD RESIN TO REMOVE SYNTHETIC CONTAMINATION FROM ARCHAEOLOGICAL BONE PRIOR TO RADIOCARBON DATING

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ABSTRACT. Museum collections are extremely valuable sources of material for ongoing research, although the conservation history of some objects is not always recorded, which can be problematic for chemical analyses. While most contamination is removed using the acid-base-acid treatment, this may not be the case for cross-linked contamination. The XAD resin protocol was implemented at the radiocarbon (^{14}C) laboratory in the Muséum national d'Histoire naturelle, and the setup was tested using known age bone samples and a consolidated Palaeolithic bone. Known age samples were consolidated with shellac or Paraloid, aged for a month, treated with or without the XAD resin and ^{14}C dated. Bone blank results showed that XAD resin was able to remove shellac, which was not the case for the ABA-only method. Results from VIRI I were more variable and VIRI F was possibly too young to show the effects of the consolidants. Two ^{14}C dates on the Palaeolithic bone after XAD treatment are statistically the same, while a sample without XAD treatment was significantly older, suggesting that the contaminant was not fully removed by the ABA-only treatment. This study demonstrates the potential of the XAD treatment to clean heritage bone samples stored in museums prior to geochemical analyses.

KEYWORDS: bone collagen, consolidant removal, contamination, radiocarbon dating, XAD.

INTRODUCTION

Bone collagen is one of the most common, yet also one of the most challenging materials for radiocarbon dating tissues from archaeological contexts. Chemical cleaning protocols usually involve an ABA treatment followed by filtration steps (Longin 1971; Brown et al. 1988; Bronk Ramsey et al. 2004), although in some cases this is not enough to remove contamination, which can be natural (humic acids) or artificial (consolidants) in origin. Identification of the contaminant can greatly aid in removal strategies and consulting the archival records can sometimes reveal which consolidants were applied. Johnson (1994) presents an overview of consolidants used from the years 1900–2000 that have been recorded in archaeological literature. While initially natural resins were the preferred choice of consolidant, this shifted to synthetic materials around the 1930s, although it also depended on the conservator in question, their experience and preferred working method. However, treatment specifics (which brand or which solvents were used) or treatments altogether were not always documented, as they were at the time considered common (Brock et al. 2018). For example, shellac shows up in the literature from 1920 to 1960, while Paraloid B72 (an acrylic resin) has only a single reference in the 1980s (Shashoua 1989), even though Johnson reports that it was widely used and was even considered the best choice for bone consolidation. Therefore, it is useful to check museum specimens for the presence of consolidants using rapid, cheap, and minimally destructive analytical techniques, such as FTIR-ATR (Fourier transform infrared spectroscopy in

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Attenuated Total Reflectance mode), before subjecting them to any expensive and more destructive chemical analyses.

We discuss here our considerations when radiocarbon dating bone samples with unknown conservation history. First, in section 1.1) FTIR-ATR quality control can be used to investigate the bone preservation (collagen content) and identify potential contamination (originating from the burial environment or consolidants). Then in 1.2), previously used consolidant removal treatments from the literature and their drawbacks. In 1.3), cross-linking between consolidant and collagen can be a problem for this removal. We tested the reliability of 1.4) XAD resin for the cleaning of artificially "aged" bone samples conserved with two common consolidants (shellac and Paraloid B72).

1.1 Identifying Contamination Using FTIR-ATR

While there is a vast library of IR spectra available, most of these enable the identification of a pure sample of conservation material. However, identifying which conservation material has been applied to archaeological bone can be difficult, as many of the molecular groups overlap with those present in bone collagen, which are usually more abundant and could mask the presence of preservatives. Additionally, it is not unusual to have a mixture of conservation materials (Derrick et al. 1999). To complicate things further, absorption bands of consolidants may slightly alter when applied to bone and after cross-linking with the collagen (Horie 2010; Law et al. 1991). As such, the peaks may not be at the exact same position, although it is unclear how far they may shift. Additionally, D'Elia et al. (2007) state that more research into the detection limit of FTIR-ATR is required. Law et al. (1991) report that contamination of a few percent was not possible to detect with FTIR. Van Klinken and Hedges (1995) mention that (in pers. comm. with Law) the presence of humics can be detected with FTIR above the 10% level, suggesting that samples without a humics signal can still contain considerable amounts of humics. To what extent this could impact the ^{14}C age depends on the age of the bone sample, as well as the age of the humics. Overall, FTIR-ATR is a useful, cheap, and rapid method to gain insight into the bone collagen preservation (Lebon et al. 2016) and check the presence of contamination, although there is considerable uncertainty over the detection limit and so potentially large quantities contamination could remain undetected in the sample.

1.2 Consolidant Removal Treatments

Bruhn et al. (2001) investigated the removal of a range of consolidants applied to known age wood by using a Soxhlet extraction with various chemicals, a treatment that has been applied to clean bone samples in other studies (e.g., Yuan et al. 2007; Ramirez Rozzi et al. 2009). While the treatment using the Soxhlet improved the ^{14}C results, it is possibly too aggressive for small and/or badly preserved bone samples, thus risking the potential loss of the entire sample.

Fedi et al. (2016) analyzed archaeological bone samples treated with Paraloid. They observed that the sharp peak at 1740 cm^{-1} visible in the FTIR-KBr spectrum of Paraloid itself was not clearly visible in the spectrum of the contaminated bone sample, which could be due to sample heterogeneity or overlap with the amide band. They found that even when four chloroform extractions were used, Paraloid was not fully removed. Other studies tested whether consolidants applied to bone can be removed; Takahashi et al. (2002) examined bone samples previously contaminated with hide glue (not successful), while D'Elia et al. (2007) contaminated bone with various substances: a waterproof pen for 8 hours, with Paraloid 72 for 8 hours and with calcite and humic acid for 48 hours at various temperatures (successful but

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young bone). Contamination with the waterproof pen was performed by completely covering the samples with the ink at room temperature for 8 hours. For Paraloid 72™, the samples were completely immersed in the contaminant for 8 hours at room temperature. For the contamination with calcite and humic acids, the samples were immersed in a water solution of the contaminant for 48 hours at 60ºC, 100ºC, and 200ºC. Van Klinken and Hedges (1995) observed that humic substance uptake by collagen can be quick (several hours), and only ninhydrin and HPLC treatments could remove the humic contamination in the case of cross-linking.

Meadows et al. (2019) looked at Mesolithic bone and antler objects, which were consolidated with unknown material. Two types of consolidant with distinct FTIR-ATR peaks were used, one of which showed peaks that suggested cellulose nitrate, while the other consolidant could not be extracted and analyzed separately. Samples were cleaned with a Soxhlet using organic solvents, followed by an ABA protocol. While the authors have no reason to question the results, they were also unable to prove that all the contaminants had been removed.

In order to more accurately replicate real archaeological material, several studies (Dee et al. 2011; Brock et al. 2017; Brock et al. 2018) artificially aged the testing medium chromosorb for one month in a climate chamber (temperature 60°C and 100% humidity). Dee et al. encountered difficulties in removing glues and adhesives, whereas Brock et al. (2018) successfully removed shellac and Paraloid but had trouble with polyvinyl acetate (PVA) and cellulose nitrates. Chromosorb contaminated with pitch tar could be almost entirely cleaned, although the archaeological samples from the Pitch Lake produced mixed results; a cranium gave an acceptable ^{14}C age, while the ^{14}C ages of the wooden objects were much older (Brock et al. 2017). While these experiments provide useful insight into consolidant removal before applying this to valuable archaeological material, the chromosorb is chemically not the same as archaeological bone (Brock et al. 2018). As such, it would be valuable to perform a similar experiment on known age archaeological bone, where contamination has the chance to cross-link to the collagen.

1.3 Cross-Linked Contamination

Cross-linking of the collagen molecule happens during and after biosynthesis (Robins 1983) and occurs through a range of chemical processes together referred to as the Maillard reaction, or non-enzymatic browning, which is a condensation (amino-carbonyl) reaction between free amino groups of proteins and sugars, although it can also happen with any other component with a carbonyl group (Maillard 1913; van Klinken and Hedges 1995), including humics. Humics (i.e., fulvic acid, humic acid and humin) can enter bone from the soil, bringing exogenous carbon into the sample, although the process of *in situ* humification of the bone itself, as a result of the Maillard reaction can similarly lead to the presence of humics (van Klinken and Hedges 1995). How to distinguish between the two types or how these interact is not well known (van Klinken and Hedges 1995; Nicholson 1998). Humic substances can cross-link to the collagen molecule, rendering the structure less susceptible to enzymatic attack (van Klinken and Hedges 1995). Unfortunately, these cross-linked humics have proved difficult to remove.

In an experiment to test the uptake of humic acids, van Klinken and Hedges (1995) found that the uptake occurred in a matter of several hours with a maximum uptake of 25%. In the light of radiocarbon dating, they tested the efficiency of, at that time current, cleaning methods and found that only HPLC purification and ninhydrin produced clean samples, while other

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methods still left humics behind in the sample. Arenella et al. (2014) used solubilised protein and humic acid (in varying proportions for 24 hours at pH 7 at a temperature of 20ºC) and observed a peak shift in matrix-assisted laser desorption-ionization (MALDI) mass spectrometry analyses, indicating that the linking of humic acids with proteins resulted in a change in mass. Other than perhaps observing such a peak shift in MALDI spectra, it is currently not possible to identify if cross-linking of contamination occurred in a bone sample in the first place, let alone quantify this. This is evidently more problematic for older and badly preserved samples.

When contamination, whether environmental or artificial in origin, has cross-linked to the collagen molecule, this can only be eliminated by breaking apart the collagen structure and releasing the contamination. Three methods currently exist to eliminate this type of contamination prior to ^{14}C dating: ninhydrin, single amino acid analysis and XAD resin, as these methods employ a hydrolysis step, breaking apart the collagen structure and releasing any cross-linked contamination. However, the sample size required for both ninhydrin (Nelson 1991; Tisnérat-Laborde et al. 2003) and single amino acid analysis is considerably large (40–50 mg of bone collagen (Marom et al. 2012; Devièse et al. 2018), while the XAD can deal with sample sizes that are normally used for ^{14}C dating (2.5–3 mg bone collagen), although smaller is also possible.

1.4 XAD Resin

Various types of XAD resins exist (Stafford et al. 1988), which are commonly used in environmental research to extract dilute chemicals from fluids, e.g., humates from fresh and marine waters. XAD 2 resin is physically and chemically stable at extremes of pH, solvent polarity, and temperatures to 250ºC. Before passing through the hydrophobic XAD resin, collagen samples are hydrolyzed in hot, concentrated HCl acid, inducing two essential changes in the sample. Humic and fulvic acids, as well as synthetic conservation materials, will polymerise and, as a result, become non-polar and able to stick to the hydrophobic XAD resin. At the same time, the collagen molecule will break down into amino acids, which are neutral to weakly polar and thus will be able to pass through the resin. The breaking down of the collagen structure is crucial in order to free any cross-linked contamination from the collagen.

The use of XAD resin for cleaning radiocarbon samples was initiated by Stafford et al. (1982, 1987, 1988, 1991), who continued with this method (Welch et al. 2012), including studies using challenging material, for example from Clovis and Pre-Clovis sites (Waters and Stafford 2007; Waters et al. 2011; Waters et al. 2015), and Kennewick man (Stafford 2014). Devièse et al. (2018) compared radiocarbon dates prepared using both HPLC and XAD resin and highlighted that these methods are currently the only ones removing environmental and museum-derived contaminants entirely, whereas other pre-treatment methods are simply unreliable in removing all contaminants.

The XAD method was never widely adopted in European radiocarbon laboratories, possibly because it is considered to be labor-intensive and time consuming compared with the ultrafiltration method (Herrando-Pérez 2021). Minami et al. (2004) compared the classical ABA extraction to the XAD method. Samples treated with a base step produced the same ^{14}C age as the XAD cleaned samples. However, samples used in this study were well-preserved, while the impact of the base step can have a larger impact on the collagen yield in badly preserved bones (i.e., low collagen yielding bones), as found by Minami and Nakamura (2000).

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They found that younger ^{14}C ages were produced by bones with low collagen yields using bone gelatin compared to the XAD cleaned syrup and recommend using the XAD method only for low collagen yielding bones as the XAD method is quite time-consuming.

Here we present the results from the initial testing phase while setting up the method in the radiocarbon laboratory in the Muséum national d'Histoire naturelle, Paris, France. The aim of this testing phase was twofold. Firstly, in order to verify that the XAD method does not leach any carbon contamination to samples, samples of known age were processed with the XAD resin and ^{14}C dated. This would also reveal whether good blanks could be obtained with this method in our laboratory, considering that the XAD method adds several steps to the protocol, which renders samples more prone to contamination with each manipulation.

Secondly, an experiment was designed to test if the XAD resin removes contamination that the classical treatment cannot remove. Samples of known age were contaminated with two types of consolidant, artificially aged in a climate chamber, subjected to a treatment with or without the XAD method and ^{14}C dated. The artificial aging aspect is crucial in order to imitate archaeological samples as well as possible, although this remains an approximation (Horie 2010). An archaeological sample that had been treated with consolidant was also included in the experiment.

MATERIAL

Known-age bone samples (VIRI I whale bone, consensus age 8331 ± 6 yr BP, VIRI F horse bone, consensus age 2513 ± 5 yr BP and VIRI H whale bone, consensus age 9528 ± 7 yr BP (Scott et al. 2010)) were used in addition to two bone blanks; an in-house bone blank (PC-14) and the Hollis mammoth bone blank (FmC = 0.0031 ± 0.0002, (n = 219), conventional ^{14}C age = 46400 ± 520 (rounded according to Stuivert and Polach (1977), with the Libby half-life of 5568 yr) from Yukon, Canada, (Martinez De La Torre et al. 2019), which was kindly supplied by Hector Martinez De La Torre. An archaeological (Palaeolithic) bone sample (SC B8 153 147) from the site of Santa Catalina, Spain, that was both visibly contaminated, as well as according to the FTIR-ATR results, was used in the testing phase of the XAD method. The monograph of the excavated material from this site stated that some bone material was consolidated with Paraloid (5% diluted in acetone) due to its poor state of preservation (Berganza Gochi and Arribas 2014). However, which samples were subjected to this treatment was not specified. FTIR-ATR analysis on this specific bone (SC B8 153 147) revealed an anomalous peak in the spectrum at 1725 cm^{-1}, which most likely originates from the Paraloid in this case.

1. Exclude leaching of XAD resin

To exclude leaching of carbon from the XAD resin to samples, 18 bone samples (13 Hollis bone blanks, 2 in-house bone blanks (PC-14), 2 VIRI I and 1 VIRI H) were collagen extracted and treated with XAD resin.

2. Test functionality of XAD resin - the ageing experiment

To verify the functionality of the XAD resin, 18 bone samples (triplicates of Hollis bone blank, VIRI I and VIRI F) were consolidated with either shellac or Paraloid B-72. Shellac is commonly dissolved in ethanol (Brock et al. 2018), while Paraloid is often dissolved in acetone (Johnson 1994).

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1. Shellac was prepared from dry, brown/orange flakes dissolved in a saturated solution in ethanol (unknown supplier, provided by the Muséum national d'Histoire naturelle, gomme laque sennelier, CRCDG no 45 by).
2. Paraloid B-72 was dissolved in a 5% w/v solution in acetone, prepared from solid pellets (Paraloid B72 Ethyl-Methacrylat copolymer Kremer pigmente GmbH & Co.KG, provided by the Muséum national d'Histoire naturelle).

Bone samples were completely submerged in either the shellac or Paraloid solution. These consolidated samples were placed in a climate chamber for artificial aging for 28 days at 50ºC with a relative humidity of 80% by Dr. Sophie Cersoy at the Centre de Recherche sur la Conservation (CRC, UAR 3224) Muséum national d'Histoire naturelle. Bone powder from these consolidated samples was first analyzed with FTIR-ATR to investigate whether or not anomalous peaks could be observed in these samples, before subjecting them to collagen extraction. There was enough collagen left to also ^{14}C date one consolidated sample (not in triplicates) without the use of the XAD resin for comparison, while a clean (i.e., uncontaminated) piece of each of these bone samples was also treated with XAD resin for comparison. The consolidants themselves were also ^{14}C dated. We expect Paraloid to give an older date, while shellac will likely give a modern age. The reason to test a young and old consolidant is that Paraloid may not have a large impact on the ^{14}C age of a bone blank but it may affect younger bone material.

METHODS

FTIR-ATR

Bone powder (1 mg) was analyzed by Fourier Transform Infrared Spectroscopy in Attenuated Total Reflectance mode (FTIR-ATR) by pressing the powder between the surface of a diamond crystal using a single reflection ATR-Golden Gate accessory (Specac) on a Vertex 70 spectrometer Bruker (Musée de l'Homme, Paris, France). Spectra were collected with a spectral resolution of 4 cm^{-1} for 32 scans in the range of 4000–370 cm^{-1}. The anvil pressure on the ATR crystal was adjusted to obtain a raw absorbance of 0.5 for the $\nu 3PO_4$ band around 1015 cm^{-1} and spectra were background corrected (Lebon et al. 2016).

Bone Collagen Extraction

Bone samples (chunks) were demineralised in 0.2 M HCl for several days (mechanical and visual checks) during which the acid was renewed several times. Samples were rinsed three times with Milli-Q, submerged in 0.1M NaOH for 20 min (if discolouration appeared, new NaOH was added for another 20 min), rinsed three times with Milli-Q, submerged into 0.1 M HCl for 10 min, followed by three Milli-Q rinses. Samples were gelatinised in weak (pH 3) HCl acid at 90ºC until dissolution, filtered using glass filter units (mesh size 10–20 μm), frozen using liquid nitrogen and lyophilised in clean (baked out) vials.

Ultra-clean XAD 2 resin, filter frits (porosity 20 μm) and empty 1 mL columns were purchased from Restek. The XAD 2 resin was cleaned in the bottle with acetone, numerous washes of distilled water and several washes of increasing molarity of HCl from 0.1 M upwards, after which it was stored in 1 M HCl.

Lyophilised collagen samples were dissolved in 1 mL of (sub-boiling distilled) 6 M HCl in 10 mL borosilicate tubes with PTFE lines caps and hydrolyzed at 110ºC for 24 hours.

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The hydrolysate was passed through pre-cleaned and pre-conditioned XAD columns. Columns were fitted with a filter frit at the bottom, filled with ±100 µL (ca. 1 cm) of XAD 2 resin slurry and covered with the top filter frit, which was pushed down to remove air bubbles. The columns were washed with 20 mL of 1 M HCl and preconditioned with 10 mL of 6 M HCl. After the sample hydrolysate had passed through, the column was washed with 1 bed volume (ca. 1 mL) of 6M HCl to collect any amino acids in the void space and this was added to the collected sample. Samples were dried in small open beakers on a hotplate in the fume hood and rinsed with Milli-Q to remove any leftover HCl by evaporation. Afterwards, samples were transferred in ~200 µL (7–8 drops) of Milli-Q to combustion tubes using glass Pasteur pipettes, frozen in the freezer and lyophilised.

Combustion, Graphitisation and ^{14}C Measurements

After adding a baked out silver strip (10 mg), samples were connected to the CO_2 extraction line in the radiocarbon laboratory (Muséum national d'Histoire naturelle). Pure O_2 (900 mbar) was added, after which samples were combusted at 900°C for 20–30 min, cleaned on the CO_2 extraction line (water trap, NOx oven fitted with copper and silver fibre wool) and volume calculated. The CO_2 gas sample was transferred to a semi-automated H_2 reduction line using iron as a catalyst. Samples were run alongside standards (oxalic acid and phthalic acid). Graphite targets were dated using the ECHoMICADAS AMS at Gif-sur-Yvette (France). Data reduction was performed by BATS software (version 47) (Wacker et al. 2010). The first few scans were discarded to eliminate possible contamination of the target with ambient atmosphere between target pressing and AMS measurement. Radiocarbon ages were calculated from $F^{14}C$ (Reimer et al. 2004), which is corrected for blank and isotopic fractionation for the samples and only isotopic fractionation for blank values. The standard deviation of the blanks is generally less than 10% and represents the counting statistics and C13H correction, which is the smaller error bar on the blank measurements in Figure 2. An overestimated standard deviation of 30% is imposed to the blank value in order to take into account a potential variability of the contaminant which could be added during the sample preparation, which is visible as the larger error bar in Figure 2. For clarification, blank values that are not blank corrected will be marked with * in the text for clarification, and the error bars on these blank values reflect only the counting statistics and are not imposed with an additional 30% standard deviation. Measurement parameters such as ^{12}C current and ^{13}CH current were checked. Time, current and isobar corrections were made prior to validation. Normalisation, correction for fractionation and blank corrections were applied for each individual run by measuring the oxalic acid II NIST standard, its $^{13}C/^{12}C$ ratio and the chemical blanks. The sample processing in this workflow without XAD (chemistry, combustion, graphitization, ^{14}C measurement) produced excellent results of the Hollis bone blank: $F^{14}C = 0.0021 \pm 0.0006$ ($N = 3$).

IRMS

Bone collagen samples (320–380 µg) of the archaeological bone (SC B8 153 147) were weighed into tin capsules and analyzed with a Thermo Scientific EA Flash 2000 coupled to a Delta V Advantage isotopic mass spectrometer. Isotopic values of all samples were measured relative to the laboratory standard alanine, which has a reproducibility of 0.3 wt% for N and 0.6 wt% for C. $\delta^{13}C$ and $\delta^{15}N$ values are reported relative to the VPDB and AIR, respectively. Analytical precision is ± 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. The atomic C:N ratios are reported in this paper, but not the stable isotope ratios.

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RESULTS AND DISCUSSION

The atomic C:N ratios of the archaeological bone collagen (SC B8 153 147) fall between 2.9-3.6 (DeNiro 1985) (3.29 and 3.32), showing that the collagen is preserved well enough to produce a reliable ^{14}C date, while the collagen yields (6.2% and 6.6%) should be between 1–22wt% (van Klinken 1999).

Detecting Consolidants with FTIR-ATR

One sample of each contaminated bone triplicate was subjected to FTIR-ATR, the spectra of which are shown together with spectra of the consolidants (in black) and a modern uncontaminated bone reference (in red) (Figure 1A and 1B). The majority of the peaks from the Paraloid and shellac either largely overlap with bone peaks or are atmospheric CO_2 derived (around 2200–2400 cm^{-1}) and not representative of any functional groups (Brock et al. 2018). Key regions in the FTIR-ATR spectra where Paraloid and shellac could be expected to be visible are 1300–1100 cm^{-1}, ~1725 cm^{-1} and 3000–2800 cm^{-1}, as is visible from the consolidants' spectra we analyzed (Figure 1A and 1B). There seems to be a small shoulder present at 1725 cm^{-1} in the spectra of the VIRI I and Hollis samples contaminated with shellac, while this is more pronounced in the case of Paraloid (Figure 1A and 1B insets). However, nothing is visible in the VIRI F sample, which is possibly related to the detection limit of the FTIR-ATR method. Both shellac and Paraloid show peaks between 3000–2800 cm^{-1} (C-H stretching), which seems to affect the spectra of VIRI I and Hollis in the case of shellac and only VIRI I in the case of Paraloid. Again, in both cases nothing is visible in the VIRI F samples. Finally, the region between 1300–1100 cm^{-1} shows no trace of these consolidants, possibly due to the overlap with the Amide III peak from the bone (~1250 cm^{-1}), thus masking the signal from the consolidant. As is mentioned in the literature (Law et al. 1991; Horie 2010), FTIR spectra of pure (consolidant) samples do not necessarily produce peaks in precisely the same place when they have been applied to or when they have cross-linked with a certain material (such as bone). Hence the difficulty in identifying the consolidant from a mixed signature (bone + consolidant).

Additionally, the difference in contamination visibility in these contaminated bone samples (between Hollis, VIRI I and F) is unlikely to be related to state of preservation, as all three samples are quite well preserved (Minami et al. 2013; Martinez de la Torre et al. 2019), although the bone structure could play a role here. VIRI F is from a horse, while VIRI I is derived from a whale, whose bone structure is more porous than terrestrial mammals, thus potentially allowing consolidants to infiltrate the bone structure more invasively. However, mammoth bone, or at least some anatomical elements, can be remarkably porous as well, and has in some cases been mistaken for whale bone. As such, the degree of porosity could help explain why the Hollis and VIRI I bone samples did show peaks in the FTIR-ATR spectra and the VIRI F bone did not. Furthermore, the peak characteristics of Paraloid and shellac are very similar and based on the FTIR-ATR spectra it is not possible to distinguish the two.

The archaeological sample from Santa Catalina had an anomalous peak at 1725 cm^{-1} in the FTIR-ATR spectra (Figure 1C), which is most likely derived from a treatment with Paraloid, as stated in the monograph. However, without this information, is it difficult to narrow down the constituent. Law et al. (1991) found that peaks at 1725 cm^{-1} with FTIR-KBr on bone samples could represent peptides (-C=O or -COOH) but also the C=O group of acetate in PV-OH at 1740 cm^{-1}. The authors also report a range of other characteristic absorption peaks for PVA and PV-OH originally from Haslem et al. (1972) and Bradbury et al. (1958), although

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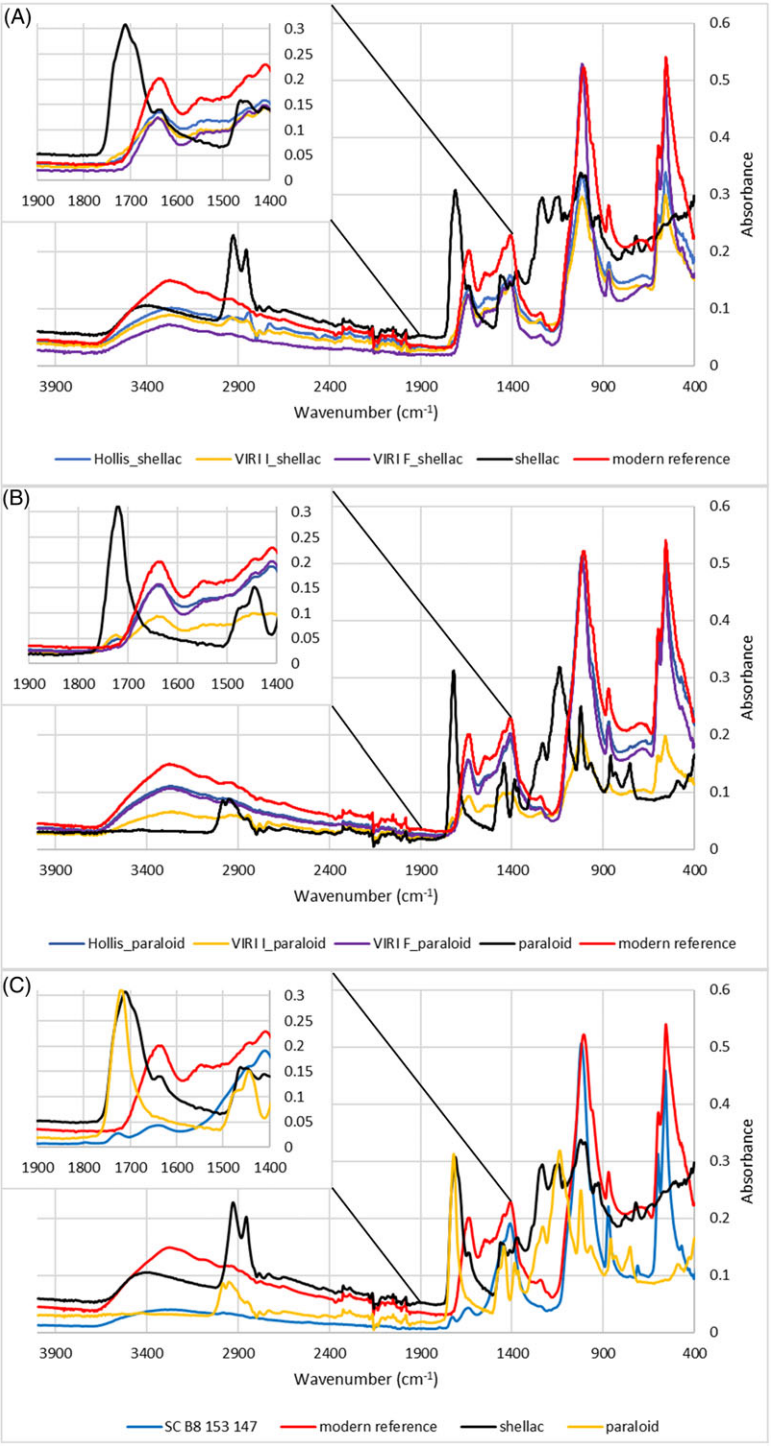

Figure 1 FTIR-ATR spectra of the bone samples contaminated with shellac (A) and Paraloid B-72 (B), and the Santa Catalina bone (SC B8 153 147) (C). The insets show the spectra around the peak at 1725 cm^{-1}.

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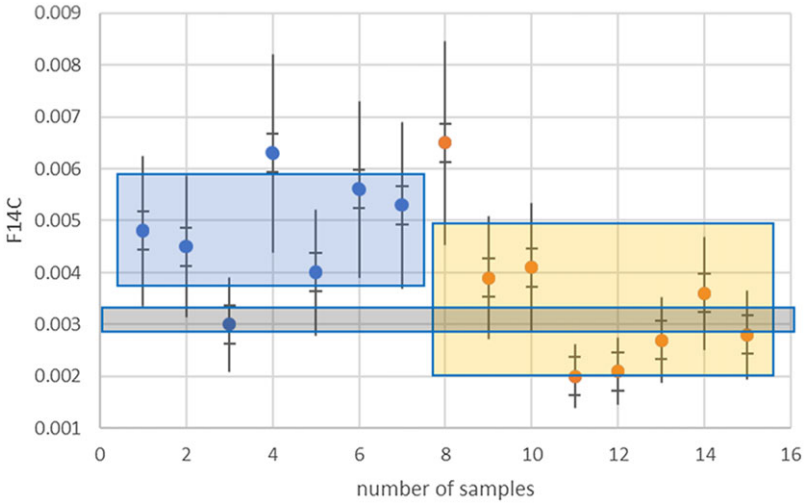

Figure 2 $F^{14}C$ results of the 15 bone blanks to test leaching of carbon from the XAD resin. Each point has a small error bar representing only the counting statistics and C13H correction, and a larger error bar representing the added 30% dispersion. The grey band represents the value reported by Martinez De La Torre et al. (2019) (FmC = 0.0031 ± 0.0002), while the blue band represents the average and standard deviation of the first sample batch, and the yellow band for the second sample batch.

most peaks are likely to be masked by the bone spectra. Derrick et al. (1999) report a whole range of polymers that could produce a strong carbonyl band in the region of 1750–1700 cm^{-1}, including polyesters (e.g., Mylar), acrylics (e.g., Acryloid), alkyds (e.g., Glyptal), poly(vinyl acetates) (e.g., AYAA), plasticised polyvinyl chlorides (e.g., vinyl storage sleeves), polyurethanes (e.g., Adiprene L-1 00), and cellulose esters (e.g., cellulose acetate). Compared to the spectra in Derrick et al., a peak at 1720 cm^{-1} may indicate shellac, Acryloid B-72 and/or oil, although the peak could similarly originate from other synthetic resins such a PVAC or Polyester 12F. Mitchell et al. (2013) found that FTIR-ATR on polymer fragments produced a peak at 1721 cm^{-1}, indicating a carbonyl stretch that can be present in cellulose nitrate, while Poly(vinyl chloride) has a peak at 1720 cm^{-1} and polyurethane has a peak at 1725 cm^{-1}. Most other characteristic peaks largely overlap with peaks that are normally present in archaeological bone. More recently, Brock et al. (2018) report FTIR-ATR peaks for different types of shellac, Paraloid, PVA and cellulose nitrate. Apart from the latter, the other three show a peak similar to our samples—Paraloid and PVA have peaks at 1720 cm^{-1} and shellac at 1710 cm^{-1}. As such, based on the literature alone, the peak at 1725 cm^{-1} in the archaeological sample from Santa Catalina could come from any of these consolidants. It is therefore crucial that future, if any, treatments are documented accordingly.

Radiocarbon Dating

The average of all 15 bone blanks that were collagen extracted and treated with XAD resin was $F^{14}C = 0.0041 \pm 0.0016$*. Figure 2 shows that the measurements in the second sample batch seem a bit better but considering the 30% error on the blanks, they are not different from the earlier measurements.

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Table 1 ^{14}C ages of the uncontaminated VIRI I and H bone samples.

Number of samples	^{14}C age (yr) BP	Consensus age
Average VIRI I *n=2*	8378 ± 53	8331 ± 6
Average VIRI H *n=1*	9680 ± 60	9528 ± 7

The VIRI I and H samples turned out to be slightly older than their consensus age (Table 1). The drying step is the moment where samples are most prone to contamination (open beakers) and any improvements in the setup could be made at this part in the protocol. We expect that enhancing the drying step could improve the reproducibility of the blanks.

Overall, in the consolidation experiment we observed that samples contaminated with Paraloid (^{14}C age of 27 290 ± 170 yr BP*) and treated without XAD produced an older ^{14}C age than samples treated with XAD (Figure 3). The opposite was true for shellac (^{14}C age of –2415 ± 20 yr BP*) contaminated samples, samples without XAD treatment produced younger ^{14}C ages than samples treated with XAD. The Hollis bone blank samples showed the largest difference in ^{14}C ages between samples that were treated with ABA-only versus ABA and XAD. As the Paraloid used in this experiment is very low but not completely free of ^{14}C, it is unlikely that the Paraloid would have made the Hollis bone appear older. Therefore, the ^{14}C age of the Paraloid contaminated sample that was treated without XAD still shows an excellent ^{14}C age, while the shellac contaminated samples show very different results. Without the XAD, the Hollis bone blank became dramatically young, while the XAD treated samples were a lot better, although we would prefer to see older blanks (45 kyr BP or older), similar to the uncontaminated Hollis samples. As such, it might be better to increase the amount of XAD resin to ensure all the contamination is removed. Additionally, ^{14}C ages from the Hollis bone blank turned out to be statistically different between treatments, e.g., the three Paraloid samples treated with XAD, the three shellac samples treated with XAD and the three uncontaminated Hollis samples (Table 4 in suppl.). Despite these ^{14}C ages not being statistically the same, the Hollis bone blank values are good (45 kyr BP or older).

The ^{14}C results from the experimentally contaminated VIRI samples are more complicated to interpret. The ^{14}C ages of VIRI I samples contaminated with Paraloid and treated with XAD were statistically the same ($\chi 2\ (0.05) = 5.99$, $T' = 2.30$), while the ^{14}C age of the sample without XAD was different ($\chi 2\ (0.05) = 7.81$, $T' = 8.84$). The ^{14}C ages of the three samples contaminated with shellac and treated with XAD were statistically the same ($\chi 2\ (0.05) = 5.99$, $T' = 0.86$), although the ^{14}C age of the sample treated without XAD was also statistically the same ($\chi 2\ (0.05) = 7.81$, $T' = 3.44$). Furthermore, we observed some variation in the ^{14}C ages of the VIRI I samples. While the XAD treated samples produce ^{14}C ages very similar to the consensus age (8331 ± 6 yr BP), the uncontaminated VIRI I sample gave an older ^{14}C age (8395 ± 35 yr BP). This is something we have seen in the uncontaminated VIRI I bone samples as well (Table 1) and it is worth considering if this variation in age could potentially be related to the turnover rate of bone collagen in large mammals such as whales. Of all intercomparison samples, VIRI I had the largest number of outliers, 10 out of 59 observations (16.9%), while this was much lower (3 out of 55, 5.5%) for VIRI H, which is slightly older, but also a whale bone (Scott et al. 2010). As such, the variation in VIRI I may not necessarily be species related but related to this (whale) individual. The radiocarbon dates of VIRI F between the different treatments are statistically the same. This is probably because this bone is too young to see any of the effects of the Paraloid or shellac. Overall, the results from the VIRI samples are difficult

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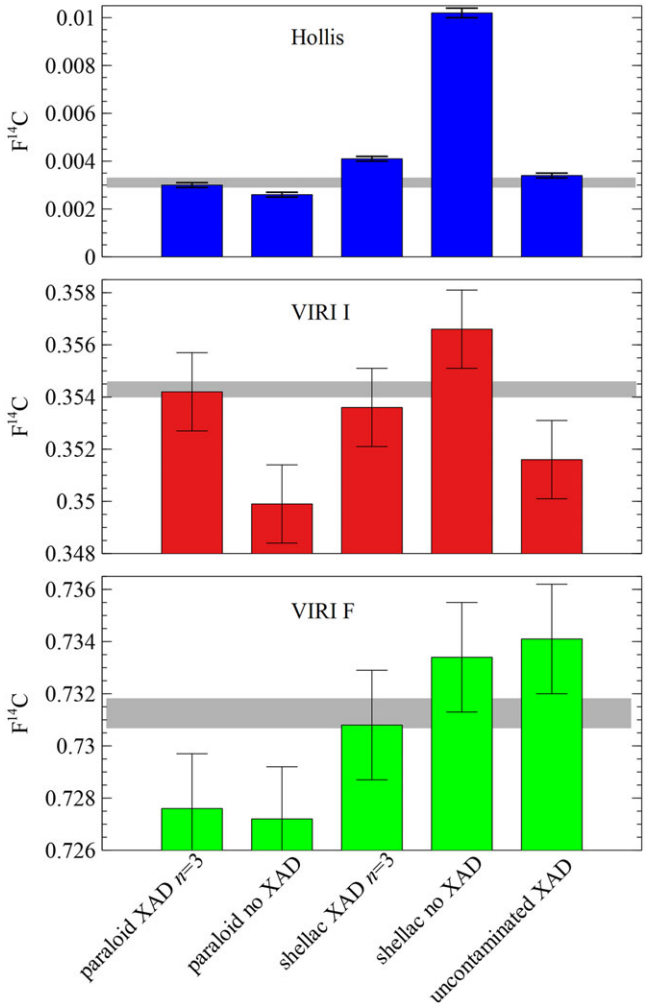

Figure 3 $F^{14}C$ results from the contamination experiment. The grey bands represent the value reported by Martinez De La Torre et al. (2019) (FmC = 0.0031 ± 0.0002) for the Hollis bone blank, and the consensus ages for VIRI I (8331 ± 6 yr BP) and VIRI F (2513 ± 5 yr BP) respectively. The Hollis uncontaminated sample is represented by 3 measurements, while there is only 1 sample for VIRI I and F.

to interpret due to their relatively young ages as well as the variation in the consensus age in the case of VIRI I.

Application to the Santa Catalina Sample

Different fractions of the Santa Catalina bone collagen were radiocarbon dated (Table 2). Two ^{14}C dates from 2 different extractions are statistically the same ($\chi 2$ (0.05) = 3.84, T' = 0.014), while one sample radiocarbon dated without XAD treatment turned out to be statistically older: 13 300 ± 50 yr BP ($\chi 2$ (0.05) = 5.99, T' = 11.79). Seeing as the contaminant makes the

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Table 2 ^{14}C results from the archaeological bone from Santa Catalina using different treatments.

	Lab number	Treatment	^{14}C age (yr) BP	±	Atomic C:N ratio	Collagen yield
Extraction 1	MUSE20033.1.2	XAD	13,070	60	3.29	6.2%
	MUSE20033.1.1.2	No XAD	13,300	50		
Extraction 2	MUSE20033.2.2	XAD	13,080	60	3.32	6.6%

sample appear older, this could point to a synthetic contaminant, which is in keeping with what was stated in the monograph (Berganza Gochi and Arribas Pastor 2014). This shows that the XAD treatment removed the contamination that the ABA-only treatment was unable to eliminate from this archaeological sample.

While it would have been preferable to increase the sample size for these experiments, it is not always feasible to obtain the right material and to destroy it for experimental purposes. Still, this study shows that the XAD treatment was able to remove contamination from the consolidants, which was not the case with an ABA-only treatment. This is at least apparent in older material, such as the Hollis bone blank (shellac) and the archaeological bone from Santa Catalina (Paraloid). However, younger material can similarly be affected by contamination from exogenous carbon, which was visible with the Paraloid in VIRI I. The contamination from consolidants may not impact the ^{14}C age of much younger material, such as VIRI F, although this would depend on the consolidants that were used. Recently, Porpora et al. (2022) published the innovative use of nanomaterial used as consolidant and its possible impact on ^{14}C dating and palaeogenetic analyses. However, the bone used to test this was quite young (^{14}C concentration of 88 pMC, Middle Ages) and the material has not been given any time to potentially cross-link with the bone collagen. Still, it is encouraging to see new avenues in the field of conservation where potential effects on radiocarbon dating are being considered.

CONCLUSION

Despite our best efforts, experiments with a climate chamber for artificial aging purposes are not infallible and it is impossible to 100% accurately replicate archaeological material that has been consolidated with conservation materials years ago. Nevertheless, the results presented here do show the usefulness of XAD resin compared with the classical ABA treatment when dealing with consolidated archaeological bones and especially the impact of young carbon on older archaeological material. Additionally, implementing and adjusting to a new method in a laboratory takes time, and improvements to existing setups should always continue in an attempt to reduce the risk of contamination. Finally, despite the detection limit, FTIR-ATR analysis can be useful as a rapid, near non-destructive technique to assess bone preservation and in some cases also to indicate the presence of consolidants, although their identification remains difficult. These experimental studies are useful as they can increase our understanding of the interaction between bone collagen and contamination, which is of great importance to archaeologists and curators.

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SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit https://doi.org/10.1017/RDC.2023.100

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