Comparison of Sandpaper and Polishing Film in Minimally-Invasive ZooMS

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Abstract

Zooarchaeology by Mass Spectrometry (ZooMS) is a rapid, cost-effective and minimally-invasive biomolecular technique that is commonly used in archaeological research of fauna identification. The developing, and minimally-invasive approach uses polishing films to produce bone powder through abrasion, which protects the fragile fauna products preserved in archaeological collections. As an abrasive material with a similar use and principle as polishing film, this experiment tested the possibility of using abrasive paper as an alternative for minimally-invasive ZooMS. Four bones and one antler were selected from previous research were sampled using four grits of sandpaper and one polishing film. The results demonstrate that sandpaper can be used for minimally-invasive sampling, and that different grit sizes affect identification quality, providing new material for ZooMS analysis.

Introduction

Zooarchaeology by Mass Spectrometry (ZooMS) is a Peptide Mass Fingerprinting method that analyzes ancient proteins through measuring the peptide of COL1 (Buckley et al. 3843), which helps archaeologists to discriminate unknown faunal remains. While ancient DNA produce results with high taxonomic resolution, it is expensive and requires a larger sample. ZooMS is cost-effective and rapid, and the developing minimally-invasive approach is able to identify animal species without destroying the bone. Past studies have compared the different minimally-invasive methods and their application on fauna artifacts, revealing that polishing film is the most suitable sampling material (Evans et al. 6). However, sandpaper has never been tested. Therefore, this project is going to test the feasibility of using sandpaper in minimally-invasive ZooMS, and explore the possibility of replacing polishing films with sandpaper. A total of 50 samples were collected from 4 bones and 1 antler. Twenty-five samples were analyzed through Matrix Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS), and 6 samples were re-analyzed once to ensure consistency in results.

Research Aims and Objectives

The aim of this research is to examine the possibility of sandpaper use as an alternative sampling material to polishing films in minimally-invasive ZooMS.

- To undertake collagen extraction from bone and antler sample through a minimally-invasive

approach;
- To identify whether sandpaper can produce identifiable spectra;
- To examine sandpaper's performance compared to polishing films;
- To compare the quality of spectra produced by different grit size.

Background

The rapid development of biomolecular methods such as ancient DNA and ZooMS have greatly increased the use of faunal remains in archaeology. Compared to other archaeological materials such as bronze and pottery, archaeofaunal remains are more fragile, less easily preserved, and are smaller in quantities. The material is also unevenly divided between geographic locations and time periods due to preservation, settlement patterns, site history, recovery practices and the focus of the archaeological research (Pálsdóttir et al. 2). The current destructive analysis in aDNA and ZooMS not only leads to varying degrees of sample damage, but also limits subsequent research because of the small quantities of faunal materials. Therefore, scholars have suggested that archaeofaunal remains should be treated as a limited resource, and sampling strategy should be carefully considered before experiment (Pálsdóttir et al. 2).

Minimally-invasive ZooMS can effectively avoid unnecessary destruction of faunal remains, preserving faunal collections. The first minimally-invasive ZooMS study using polishing film sampling sticks was published by Kirby et al. in 2013, demonstrating the particles generated from friction can be identified using Peptide Mass Fingerprinting. A case study conducted by McGrath et al. in 2019 identified archaeological bone via different ZooMS methods (original bag, forced bag, eraser, destructive) and aDNA, and examined how each method performed. A recent study by Evan et al. has further analyzed forced-bag, eraser, and coarse vs. fine grained polishing films, and suggest the coarse grained polishing films produced best results (6). As an abrasive similar to polishing film, sandpaper has never been tested, but the similar properties of the two materials suggest that sandpaper could be used for minimally-invasive ZooMS analysis.

Material and Methods

Sampling Materials

Four bone samples with known species ID were selected from the SFU database project, and one antler was collected from UBC Laboratory of Archaeology (LOA) fauna collection (see Table 1). The antler sample was not given ADaPT lot number. All of the SFU Database samples have been previously analyzed using destructive methods.

Sample	Type	Provenience	Original ZooMS Species ID
A455	Bone	SFU Database	Harbour Seal (*Phoca vitulina*)
A456	Bone	SFU Database	Fur Seal (*Callorhinus ursinus*)
A457	Bone	SFU Database	Canadian beaver (*Castor canadensis*)
A461	Bone	SFU Database	Black-tailed deer/mule deer (*Odocoileus hemionus*)
Antler 174M	Antler	LOA collection	White tailed deer (*Odocoileus virginianus*)

Table 1 Information of the sampling materials

Grit size refers to the size of the particles of abrading materials embedded in the sandpaper/polishing films (Grainger Editorial Staff). However, different standards have been established worldwide, such as CAMI, FEPA, and diameter. In North America, the CAMI standard is used (Grainger Editorial Staff). For this experiment, we chose 40, 240, 600, 1000 grit size sandpapers of CAMI standard, and 30um polishing films to make sampling sticks. According to the grit size chart in Figure 1, 30um polishing films is equivalent to CAMI 360, making it coarser than 600 and 1000 grit but finer than 40 and 240 grit sandpaper. All materials are made of Aluminum Oxide, with PSA adhesive on the back.

Grade	Description	CAMI	FEPA	Diameter	Used for
Ultra Fine	Most delicate abrasives	800 or 1000	P1500, P2000 or P2500	8.4-12.6 micrometers	Final sanding and polishing thick finishes
Super Fine	Slightly wipes away patches/small inconsistencies but not strong enough for removal	400, 500 or 600	P800, P1000 or P1200	15.3 to 23.0 micrometers	Final wood finishing
Extra Fine	Slightly less fine and more abrasive than Super Fine	360 or 320	P400, P500 or P600	25.8 to 36.0 micrometers	Initiative methods for wood polishing
Very Fine	The least fine of the micro abrasives	240	P240, P280, P320 or P360	40.5 to 58.5 micrometers	Sanding finishes between consecutive coats and drywall and wood

Grade	Description	CAMI	FEPA	Diameter	Used for
Very Fine	A coarser material than Very Fine under the micro abrasives	150, 180 or 220	P150, P180 or P220	190 to 265 micrometers	Sanding on bare wood
Fine	Cannot remove varnish or paint on wood	100 or 120	P100 or P120	115 to 162 micrometers	Preparing wood for finishing, cleaning plaster and removing water stains on wood
Medium	Medium to coarse surface texture after sanding	80	P60 or P80	190 to 265 micrometers	Sanding bare wood to prepare it for removing varnish and final finishing
Coarse	Has the ability to remove material rapidly	40, 50 or 60	P40 or P50	336 to 425 micrometers	Wiping away a layer of debris or finish with minimal effort
Extra Coarse	Quickens the removal of most materials rapidly	24, 30 or 36	P12, P16, P30 or P36	530 to 1815 micrometers	Initial efforts in hardwood floor sanding

Figure 1 Sandpaper grit size chart (Grainger Editorial Staff, 2021)

Sampling

Polishing films (grit size 30um) and sandpapers (40, 240, 600, and 1000) were used to make polishing films according to Kirby et al.'s 2013 protocol (237-238). Each bone/antler was

sampled two times using 5 groups of treatments. The label of each sample constituted of three parts: sample name, a/b to indicate the side of the bone that was sampled, and 40/240/600/1000/P indicated the treatment used. For example, A455.b240 suggest the bone A455 was sampled using 240 grit sandpaper on spot b. Extraction blanks were included for each treatment.

To sample a piece of bone or antler, the stick was rubbed against the surface until it had visibly collected bone powder or until scratch marks were created. Sampling sticks were stored in clean tubes after sampling to avoid contamination. Photographs were taken before and after the sampling to document the locations of each treatment (see Figure 2). A total of 50 samples were collected.

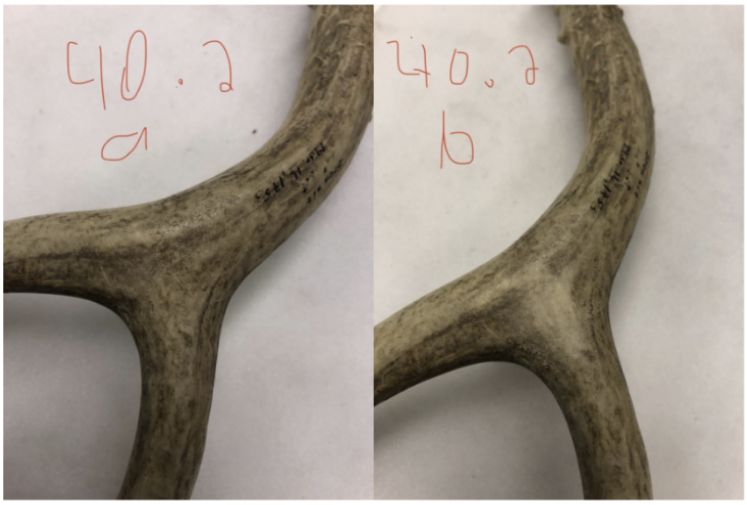

Figure 2 Sampling antler using 40 grit sandpaper, before (left) and after (right)

ZooMS Analysis

The minimally-invasive ZooMS experiment was conducted following ADaPT protocol. After sampling, the sticks were immersed in Ambic to gelatinize the bone collagen. Then, 1ul of trypsin was added to each sample to cut the collagen peptides into fragments. After incubating the samples overnight, 1ul of 5%TFA was added to deactivate the trypsin. The solution was ziptipped using C18S tips to purify the collagen peptides. A total of 25 samples were selected and spotted onto a 384 well MALDI plate, and were run on a MALDI-TOF-MS at University of York in the UK. Samples A455.bP, A461.a240, A461.a600, A461.a1000, A461.aP, and Antler.bP were rerun to test the consistency of the results. All spectra were analyzed and recorded using mMass and Excel (Niedermeyer & Strohalm 1). The ZooMS reference database comes from UBC ADaPT Facility (Buckley et al. “Species Identification by”; Buckley et al. “Distinguishing”; Buckley et al. “Species Identification to”; Buckley et al. “Species Identification and”; Buckley et al. “Species Identification and Decay”; Buckley & Collins; Kirby et al.; McGrath et al.; Welker et al.).

Results

Table 2 provides the taxonomic identifications for all samples analyzed in both runs. In the 1st run, 19 of the total 25 samples could be taxonomically identified to the species level: 12 samples matched with their original species IDs, 7 samples were given identifications that might not match with original IDs. Six samples, including all of the 40 grit sandpaper samples and A461.a1000, could not be identified. All extraction blanks produced empty spectra, suggesting that systematic laboratory contamination was not present. The results in the 2nd run matched with the 1st run, further validating the identifications.

ADaPT #	Original ID	Minimally-invasive ID
A455. b240, 600, 1000, P	Harbour Seal (*Phoca vitulina*)	Grey/Harp/Harbour Seal (*Halichoerus grypus, Pagophilus groenlandicus, Phoca vitulina*)
A457. b240, 600, 1000, P	Beaver (*Castor canadensis*)	Beaver (*Castor canadensis*)
A461. a240, 600, P; b240, 600, 1000, P	Black-tailed deer /mule deer (*Odocoileus hemionus*)	white tail deer, domestic sheep (*Odocoileus virginianus, Ovis aries*)
Antler.b240, 600, 1000, P	white tail deer (*Odocoileus virginianus)*	white tail deer (*Odocoileus virginianus)*

Table 2 Taxonomic Identification for analyzed samples

Discussion

Efficacy of Methods

To assess the quality and performance of each treatment, spectra from the same sample were visually compared.. Although some methods had more noise than another, or had significantly more non-diagnostic peaks, the overall spectra were similar to each other. Therefore, the difference between spectra is not necessarily due to different treatments, but more likely due to other steps of the experiment, such as collagen purification or MALDI-TOF-MS. Figure 3 shows the spectra of A461.b, and it can be seen that there is no significant difference except for the slightly higher noise of 240 and more peaks of P.

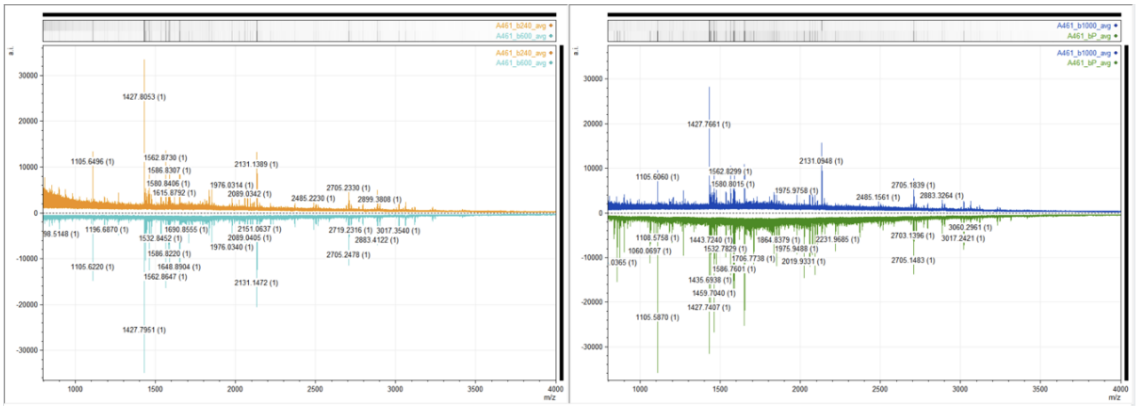

Figure 3 ZooMS spectra for A461.b (Top left: 240; Bottom left: 600; Top right: 1000; Bottom right: P)

The results of SFU Reference Project samples (A455.b, A457.b, A461.a, A461.b) were compared to their original spectra, produced through destructive methods. The destructive A457 generated the same peaks as the minimally-invasive samples. The destructive A461 produced a high 1593 peak (marker C) and 3043.4/3059.4 (marker G1/G2), which are decisive peaks in discriminating *Odocoileus hemionu*s. These missing peaks in the minimally-invasive samples led to the incorrect species ID of white-tailed deer/domestic sheep. A455 demonstrates that destructive methods are not always better than minimally-invasive, as it did not generate marker C or a clear F2 peak (2885.3), the two important peptide markers that are used to differentiate species.

To further explore the quality of spectra for each method, the number of diagnostic peaks were counted and recorded (see Table 3 and Figure 4). In this study, the definition for "diagnostic peaks" are the peaks that are picked by mMass and matched with the twelve peptide markers in the Buckley et al. article (3847) for species identification. Rerun samples and non-picked peaks were not included in the statistical analysis. Although samples A461.a and A461.b do not match with their original IDs, their peaks were still included since they were identified to one species.

Table 3 shows the number of diagnostic peaks for each method on each peptide marker. Polishing film had the best performance in species identification, followed by 600, 240, and 1000. The polishing film method was particularly effective in identifying peptide marker A1 and A2 compared to other methods, a marker that can assist narrowing down the ZooMS ID. In Figure 5, each material is arranged according to their fineness on the x-axis, and the results form a curve. The curve illustrates no positive correlation between the fineness of sampling material and the number of diagnostic peaks. In fact, I hypothesize that fine-grained materials may result in insufficient bone powder being produced and not sticking to the sampling sticks. This hypothesis conforms to the findings of Evans et al. that coarse materials can be used more effectively for minimally-invasive ZooMS analysis (6).

Sandpaper Experiment Total Diagnostic Peak Count by Peptide Markers													
Method	**P1**	**A1**	**A2**	**B**	**C**	**P2**	**D**	**E**	**F1**	**F2**	**G1**	**G2**	**Total**
40	0	0	0	0	0	0	0	0	0	0	0	0	0
240	5	0	0	5	5	3	5	3	5	5	2	4	42
600	5	1	2	5	5	3	5	4	5	5	2	4	46
1000	4	1	0	4	4	2	3	3	4	4	1	3	33
P	5	3	3	5	5	3	4	4	5	5	2	4	48

Table 3 Sandpaper experiment total diagnostic peak count by peptide markers

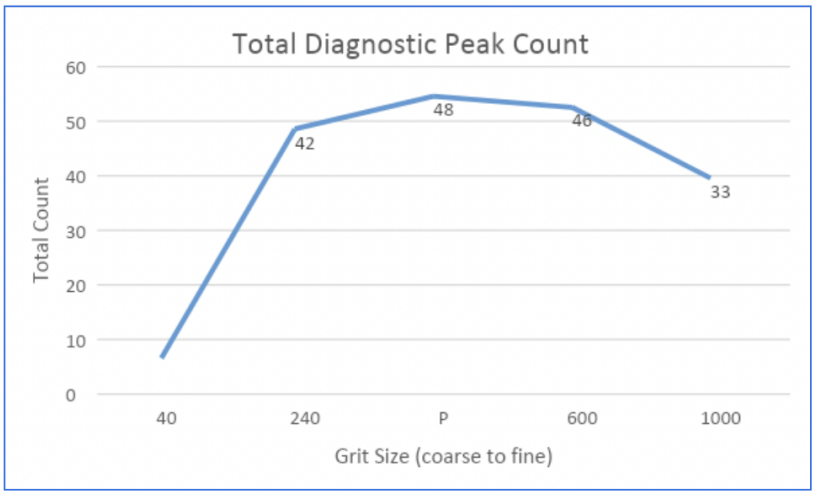

Figure 4 Total diagnostic peak count for each method

Invasiveness of Methods

While all of the methods tested were minimally invasive to the bone or antler, they did create different levels of damage to the surfaces. During the sampling process, 40 grit sandpaper formed a larger amount of bone powder than other grits, but the powder rarely stuck to the sandpaper. It also changed the color of the antler by rubbing off the outer layer (see Figure 2). The 240 grit sandpaper left scratches on the bones, but successfully generated bone powder on the sandpaper (see Figure 5). The 600 grit sandpaper created a pink stain on one of the bone samples, which might damage or contaminate the original sample (see Figure 6). The 1000 grit and polishing films both created minimal marks on surfaces, however more observation is required under the microscope (see Figure 7). In addition, compared to polishing films, the toughness of sandpaper made it difficult to bend, which affected the quality of sampling sticks, and caused the material to easily fall off during peptide extraction.

Figure 5 Sampling antler using 240 grit sandpaper, before (left) and after (right)

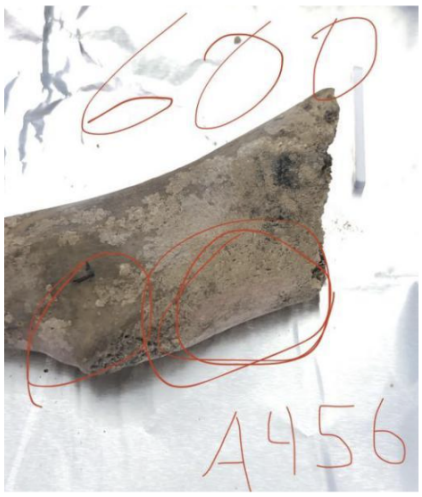

Figure 6 Sampling antler using 600 grit sandpaper, before (left) and after (right)

Figure 7 Sampling antler using 1000 grit sandpaper, before (left) and after (right)

Limitations

When designing this experiment, many variables were not considered, such as the force applied to the sticks when sampling, and the times of rubbing a bone. Since the amount of bone powder produced will directly affect the quality of the ZooMS spectra, future experiments should control the number and intensity of rubs. Other grits of sandpaper (e.g., 400, 800) and polishing films (12, 15um) should be tested to compare the results. In addition, the species of samples selected must not be unknown, so that the similarity between the destructive and minimally-invasive ZooMS results can be compared.

Since sandpapers are tougher than polishing films, large sticks can be difficult to reach the bottom of Eppendorf tubes and require forceful pushing, which could potentially dislodge parts

of the paper inside the tubes. During supernatant transfer to EXT tubes, a number of papers were no longer adhered to the stick, potentially releasing more glue chemicals into solution. This issue could be resolved by firmly pressing the paper/film to the stick to ensure full adhesion. Sandpaper also seems to soak up ~25ul of supernatant, leaving no supernatant in the SE tubes. More AmBic solutions should be added in the future to prevent this issue.

Conclusion

This experiment illustrates that sandpaper can successfully yield identifiable ZooMS spectra, providing a new sampling material for fauna identification in archaeological research. The total diagnostic peak counts show 240 and 600 grit sandpaper and polishing films performed significantly better than 1000 grit sandpaper, suggesting that the grit size is not positively correlated with the spectra quality. Overall, polishing film is the most suitable sampling material for minimally-invasive ZooMS due to its minimal scratches on fauna remains, stability during the experiment, and material features. However, 600 grit sandpaper can also be used as an inexpensive alternative when necessary.

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