A novel approach to the non-destructive identification of archaeological bones using peptide mass fingerprinting.

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Introduction

Since 2009 work from Buckley et al allowed for the identification of bone fragments through analysis of the protein collagen, labeling it Zooarchaeology by Mass Spectroscopy (ZooMS). This resilient biomolecule which can survive in the archaeological record up to 3.4 million years (Rybcznski et al. 2013), can be extracted and sequenced using Mass Spectrometer, with varying species providing a range of collagen sequences.

Until recently this methodology has been limited by it's destructive nature, relying on acid extraction from chipped or broken bone (Doorn et al 2011). This study set out to remove the destructive acid stage of the analysis and replace it with a non-destructive method of collagen extraction utilizing electrostatic.

Producing a non-destructive method has significant value in the world of archaeological species identification, not only does such work allow for wider spread analysis of bone samples, it also makes access to museum samples significantly simpler, and as such allows for a rage of more complex research questions to be asked (English Heritage 2013).

Materials and methods

The collagen extraction procedure took place through the rubbing of a PVC eraser against archaeological bone where the eraser shavings are then collected and placed into an Ammonium Bicarbonate solution to transfer the collagen from the shavings into solution.

The collagen from this solution has the enzyme trypsin added to cleave the protein at specific points and prepare it for Mass Spectrometer analysis. Following overnight incubation to allow for the trypsin to activate, the collagen is extracted from solution using C18 Zip Tip Solid Phase extraction, the process of which then allows the collagen to be isolated and spotted onto a Mass Spectroscopy plate for analysis .

This procedure was tested on 20 archaeological samples from the Roman site of Teffont, Wiltshire, and samples from the Yorkshire Museum which had been dredged from the North Sea. The majority of these animal remains had remained unidentified prior to analysis.

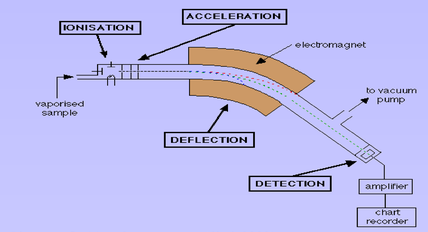


Diagram of a Mass Spectrometer (Clark 2000)

Results & Discussion

From the 20 samples analyzed, all of which were repeated, significant trends were identified within the data, the nature and consequences of which shall be explored here along with preliminary discussion as to the reason behind this.

Successful reproducible species identification was achieved for five of the samples analyzed, these were those preserved in a marine environment, along with one recently cut bone. As such we hypothesize that these samples had higher levels of endogenous collagen preserved within the bone, therefore making extraction an easier process. Those remaining samples were either contaminated or provided collagen yields to low to produce reliable results.

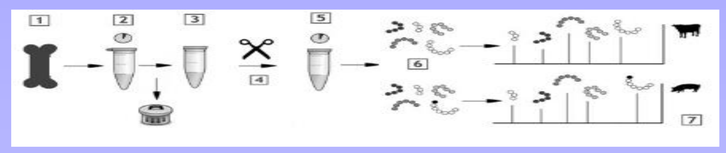
This can be explained through appreciation of the Triboelectric effect, the principal behind the extraction of the collagen. The difference in electronegativity between Collagen and PVC creates a large potential charge when the two materials come in contact (Appalachian State University 2012), and as such the following equation can be used to appreciate the relationship between the two substances:

$$\left(Charge\ affinity\ of\ collagen - (Charge\ affinity\ of\ PVC)\right) \times Friction\ of\ energy\ per\ cm^2 = Charge\ transfered\ per\ cm^2$$

(AlphaLab Inc. 2004)

The lower the amount of endogenous collagen present within a bone sample the greater the overall charge transferred is required to be, given this is dependent on both friction and the charge affinity of the substances in use we hypothesize that samples which returned unsuccessful results contained less collagen and required more friction than was provided during the experimental phase of this study.

As such to increase the overall proportional success of this methodology an increase in overall charge transferred is required. This can be achieved through both the replacement of PVC with a substance that maintains a greater charge affinity and the increase of friction energy. The increase of these variables will allow for higher collagen yields from archaeological bone containing low levels of collagen and the eventual successful identification of samples using ZooMS as demonstrated by five of the samples considered in this study.



A visual representation of the electrostatic extraction method Adapted from Doorn et al 2011: 1) Bone is rubbed by eraser to produce rubber shavings 2) Shavings are amerced in Ammonium bicarbonate and incubated for an hour at 65°C in ammonium bicarbonate buffer 3) supernatant tis extracted and placed into new eppendorf tube, 4) Trypsin added to sample to cleave the protein 5) sample incubated overnight at 37 °C 6) Peptides with varied masses are obtained 7) These variations are visualised and can be assigned using mMass.

Conclusions

This research has opened the door into further non-destructive species analysis utilizing collagen mass peptide fingerprinting, and provided a framework to increase overall reliability of a promising method. Furthermore this study has demonstrated the capacity for archaeologists to harness the electronegative nature of collagen to use as an extraction method, providing future possibilities into other biomaterials such as parchment, ivory, leather and teeth.

It is suggested that this methodology provides a bridge between the destructive analytical processes currently available for small bone fragments, and the low certainty visual identifications made on bone fragments, with a cheap, non-destructive method of analysis.

a) Sample A3 prior to collagen extraction b) sample A3 post extraction, with no visual damage occurring to sample, Author 2013

Literature cited

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Acknowledgments

Thanks for this research go to Prof. Matthew Collins, Dr. Sarah Fiddeyment and Prof. Norman Jackson for their assistance . This poster was produced using a template from http://colinpurrington.com/tips/academic/posterdesign © Template copyright Colin Purrington.

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