Multiple Techniques Confirm Collagen Remnants in Fossil Bone

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THE COLLAGEN CONTROVERSY

Detections of original organics in fossils continue to grow. Once thought of as soft parts preserved as mere impressions, new techniques reveal original biomaterials including whole tissues that persist in ancient and fossil samples of various taxa, including dinosaurs.

In 1916, Barnum Brown described a *Corythosaurus casuarius* with skin and tendon structures.[1] Modern techniques including mass spectrometry specify similar structures as consisting partly, primarily, or entirely of original organics, consistent with preserved collagen. Examples include light micrographs of blood vessels in *T. rex* and *Triceratops horridus*,[2] protein sequence in *Brachylophosaurus canadensis*,[3] SR-FTIR mapping of protein signatures in a Jurassic *Lufengosaurus*,[4] and pliable extracellular bone matrix in the mosasaurid *Prognathodon*,[5] *Allosaurus fragilis*, and Jurassic *Apatosaurus*.[6] However, artificial decay of bone collagen suggests collagen should have extinguished in Pliocene strata.[7]

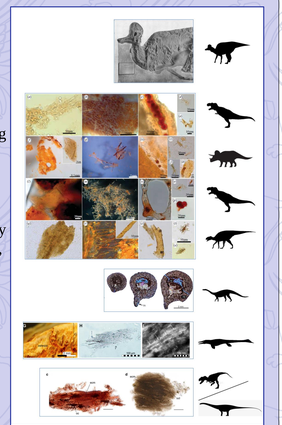

Fig. 1. Light micrographs and other images accompany dozens of molecular technues that detect collagen remnants in fossil bone. See text for references.

The aim of this study was to use novel techniques in parallel to explore the possibility of collagen preservation in fossil bone samples. Techniques included protein sequencing, second-harmonic generation (SHG) imaging, and accelerator mass spectrometry (AMS).

LC-MS/MS

Tandem Mass Spectrometry

Protein sequencing is the gold standard of collagen identification in ancient samples including bone. We sought to establish the usefulness of SHG imaging (see next panel) on fossil bone by first establishing its applicability to more recent bone.

Therefore, SHG imaging and liquid chromatography tandem mass spectrometry (LC-MS/MS) were performed on Medieval human rib bone NP73_34_81, skeleton 101, excavated 1971 from Norton Priory, UK.

Fig. 2. Norton Priory, UK. A) Photograph of monastic structures from Europe's largest monastic site. B) One of hundreds of Medieval skeletons on display at Norton Priory Museum and Gardens, UK.

We published the procedures and results in 2017.[8] They showed the presence of human collagen α-1 human collagen α-2 in this roughly 500 year-old bone sample.

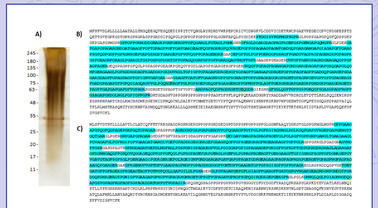

Fig. 3. Collagen sequence in Medieval human rib bone NP73_34_81 from skeleton 101. A) SDS PAGE of ~700-year-old human bone protein extract still shows a visible protein smear, consistent with endogenous collagens. B) NP73_34_81 had a 59% sequence coverage of the human collagen alpha-I chain. C) NP73_34_81 had a 65% sequence coverage of the human collagen alpha-II chain.

SHG

Second-Harmonic Generation imaging

SHG confocal microscopy uses a 920nm laser to target type I collagen fibres. Prior results demonstrated that SHG reveals decayed collagenous structures in ancient bone.[8] New results shown in Figure 4 extend SHG to three Mesozoic samples. ImageJ was used for image processing.

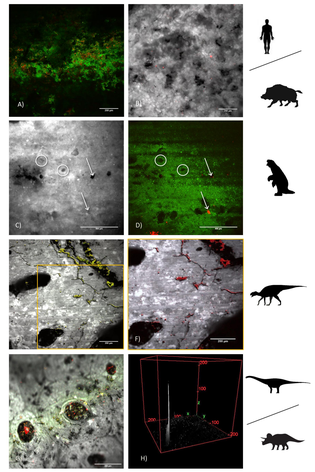

Figure 4. SHG Images of ancient and fossil bone. Red shows collagen fibre remnants and green shows autofluorescence of organic ring structures. A) Red collagen remnants in Medieval *H. sapiens* rib NP73_34_81. B) Red collagen image surface reflectance image of Roman Era *Sus scrofa* jaw XA102-2001-98 C) Reflectance of Ice Age *Megatherium* limb EHRC90002 compares surface features with SHG from the same area in D). E) SHG, autofluorescence and reflectance overlay, and F) SHG and reflectance overlay of Hadrosaur femur GDFM14.001 shows collagen in bone recesses. G) SHG, autofluorescence, and reflectance overlay of *Diplodocus longus* limb CM00094 reveals probable mineral crystals in red. H) SHG Z-stack of *Triceratops* horn core HCTH06[9] rendered 3D shows collagen signal.

AMS

Accelerator Mass Spectrometry

AMS reaches the sensitivity and resolution required to measure $^{14}C/^{13}C$ ratios. Bone collagen extracts are typically used for AMS radiocarbon dating, but some labs will measure the $^{14}C/^{13}C$ ratio also from the mineral (bioapatite) fraction.

Two commercial labs were used to test for independent collagen extraction from Mesozoic bone samples.[10] Taxonomies were withheld from submission forms in order to inhibit possible bias. Eight Cretaceous and three Jurassic dinosaur bone results were plotted in Fig. 5 as uncalibrated percent modern carbon (pmC). All results showed $^{14}C/^{13}C$ ratios greater than instrument background blanks. Research focused on isotopic signatures of primary organics and not carbon ages. Thus, AMS results from bioapatite fractions were also obtained for bones with too little collagen for dating.

Three of the 21 total AMS results show ^{14}C in collagen extracts. Fig. 4 shows SHG signals in the same three specimens. Six results show ^{14}C in bulk extracts, which mix organic (collagen) and mineral bone fractions. A lack of correlation between pmC and fraction suggests that $^{14}C/^{13}C$ can occur in any fraction. These results are consistent with the retention in fossil bone of original biological components.

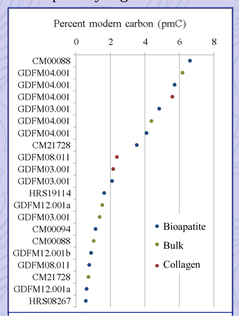

Fig. 5. 21 AMS measurements of ^{14}C expressed as percent modern carbon in 11 Mesozoic bone specimens. GDFM14.001, GDFM12.001a (a fragment from HCTH06), and CM00094 present possible collagen, as Fig. 4 shows. See Abbreviations key below for repository names. CM00088 is Morrison Fm. *Stegosaurus*. GDFM 03.001 is Hell Creek Fm. Hadrosaur tibia. CM21728 is Morrison Fm. *Diplodocus*. GDFM08.001 is Hell Creek *Triceratops* femur. HRS19114 is Lance Fm. of unknown taxon, and HRS08267 is Lance Fm. *Edmontosaurus annectens*.

CONCLUSIONS

Three logical options present themselves as possible explanations for collagen in fossil bone samples:

1. The biomaterial detected is not collagen, or is not endogenous collagen.
2. Collagen decays at a rate orders of magnitude slower than artificial decay studies show.[7]
3. The biomaterial detected is collagen, but was buried orders of magnitude later than prevailing age assignments for Mesozoic fossils.

Results from SHG and AMS, in conjunction with the literature, effectively eliminate option number one. More research will be required to eliminate other options. Future studies aim to acquire more results from AMS, SHG, and other techniques in an effort to explore geographic and stratigraphic ranges for fossil collagenous remnants.

References

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Abbreviations key:

NP = Norton Priory, Runcorn, UK
XA = (Hallaton) U. of Leicester, Liecestershire, UK;
EHRC = Earth History Research Center, Keene, TX
GDFM = Glendive Dinosaur and Fossil Museum, MT
CM = Carnegie Museum, Pittsburgh, PA
HRS = Hansen Research Station, WY

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