Some strengths and weaknesses of the polymer shield explanation for soft tissue fossils

Brian Thomas, Stephen Taylor, and Kevin Anderson

The presence of short-lived soft tissue in fossils has proven challenging for uniformitarians to explain. Wiemann and co-authors[1] describe a mode of preservation that may help explain the presence of primary protein remnants in fossil biomineralised tissues, including scales, teeth, eggshell, and bone. They showed results consistent with peptide cross-linking that forms N-heterocyclic polymers early in diagenesis.

Advanced Glycoxidation End-products (AGEs) and Advanced Lipoxidation End-products (ALEs) are a heterogeneous group of water-insoluble compounds generally formed by oxidation reactions. AGEs and ALEs resist water, chemicals, and microbes. They supposedly shrink-wrap adjacent proteins or proteinaceous remnants to shield them over deep time. The researchers summarized this preservation mode by saying:

> "The generation of brown-stained proteinaceous material, and subsequently non-proteinaceous AGEs [Advanced Glycoxidation End-products] and ALEs [Advanced Lipoxidation End-products], provides an explanation for the apparent anomaly of widespread morphological and molecular preservation of soft tissues in fossil vertebrate hard tissues. Both AGEs and ALEs exhibit hydrophobic behavior due to the chemical character of their crosslinks, which in turn shield adjacent peptides from hydrolysis. Thermo-oxidatively induced, intensive crosslinking of proteins results in hydrophobic, reinforced AGE/ALE scaffolds resistant to microbial digestion. This explains the preservation of fragile soft tissues in certain chemical environments through deep time."[1]

They offer two independent lines of evidence to support the model. Firstly, organics from both artificially matured and fossil tissues show brown staining. Secondly, both share Raman spectral characteristics. These include an increase with artificial or real age of a N-rich heterocyclic stretch peak and of a relative decrease in both Amide III and Amide II peaks that signal diminishing protein.

There is a biochemical basis for the claims of this study, which should be incorporated in future discussions of protein preservation. However, the researchers overstate their case. Their preservation model has several shortcomings and fails to adequately explain all the dinosaur tissue data.

Protein polymerisation: strengths

Experimental data connect artificially aged with actually old bone

Wiemann *et al.* have raised the bar of rigour in the defence of a protein preservation concept. They accept the robust literature that demonstrates and characterizes primary organics in fossils[2] and indeed admit original proteinaceous remnants in their fossils, including Jurassic sauropods. Explaining these fossil features within a multi-million-year time frame, however, is no easy task. Weimann and her coworkers at least demonstrate some originality in their proposal and offer two lines of experimental evidence in support.

Indeed, the polymer shield concept may help explain some published soft tissue descriptions. For example, one research team showed FTIR spectra of blood vessel-like structures in Triassic reptile bones.[3] Both Raman and FTIR spectroscopy are infrared-based indicators of vibrational modes of specific molecular bonds. Ideally, more Raman spectra could be obtained from other soft tissue fossils to compare AGEs, but even collecting FTIR spectra would allow useful comparison. These researchers ascribe an increase in FTIR peak heights, as shown in their figures 5(f) and (g), to "amino acid residues and lipid structures". These peaks may indicate AGEs or ALEs. Surmik *et al.* suggest a goethite micro-coating as a sort of thin mineral shield.[3] More spectroscopic studies could look for some combination of mineral and AGE soft tissue coatings.

Weimann *et al.* also offer a novel suggestion that brighter oxidative sedimentary matrixes have a better chance of forming the AGEs that make fossils darker. They reason that oxygen-rich burial environments should increase oxidation rates, AGE production, and thus preservation. They wrote:

> "In identifying brown vertebrate hard tissue fossils in light coloured (oxidative) sediments as a target, our observation provides a first field guide to the search for endogenous soft tissues in fossil vertebrate remains as a basis for addressing a range of evolutionary questions."[1]

Possibly brighter sediments contain a higher ratio of soft tissues in fossils, but this needs to be tested, not assumed. Other results have shown soft tissues in dark-coloured (considered reducing) sediments,[4] which negates the key feature of their field guide. Nevertheless, a field guide that could isolate fossils with higher potential of

containing preserved primary proteins is a worthwhile goal.

Protein polymerisation: shortcomings

N-heterocyclic polymers may be absent from some specimens

Wiemann and coauthors report darkened soft tissue samples from demineralised vertebrate hard tissues including diplodocid, *Allosaurus*, and *Apatosaurus* bone and *Psammornis* and *Heyuannia* egg shell. Their model, though, fails to account for numerous reports of whitish soft tissue fossils.

Such white endogenous tissues suggest a lack of the N-heterocyclic polymers that are critical to the model. For example, colour images of soft tissue remnants from decalcified *Tyrannosaurus* femur published in 2005 show pale connective and vascular tissues.[5] Admittedly qualitative in nature, the darkening expected from the polymer shield model is not readily apparent in colour images of soft tissues reported from decalcified moa, mammoth, mastodon, *Tyrannosaurus*, and *Triceratops* bone.[6] Blood vessels extracted from some fossil have been described as 'transparent', and interstitial fibrous tissues as having 'natural' (which means life-like or primary, not diagenetic) pigmentation.[6] Tissues from the *Brachylophosaurus* specimen "show the presence of white fibrous matrix that autofluoresced under ultraviolet light, consistent with collagen".[2] The white matrix described in these reports does not match the brown colour that results from reactions forming AGEs. These reports suggest a need to continue to develop and test alternative preservation models.

Reactions forming AGEs will decrease the elasticity of tissues.[7] Wiemann and co-workers also noted a specific texture to the tissue. They referred to darkened N-heterocyclic

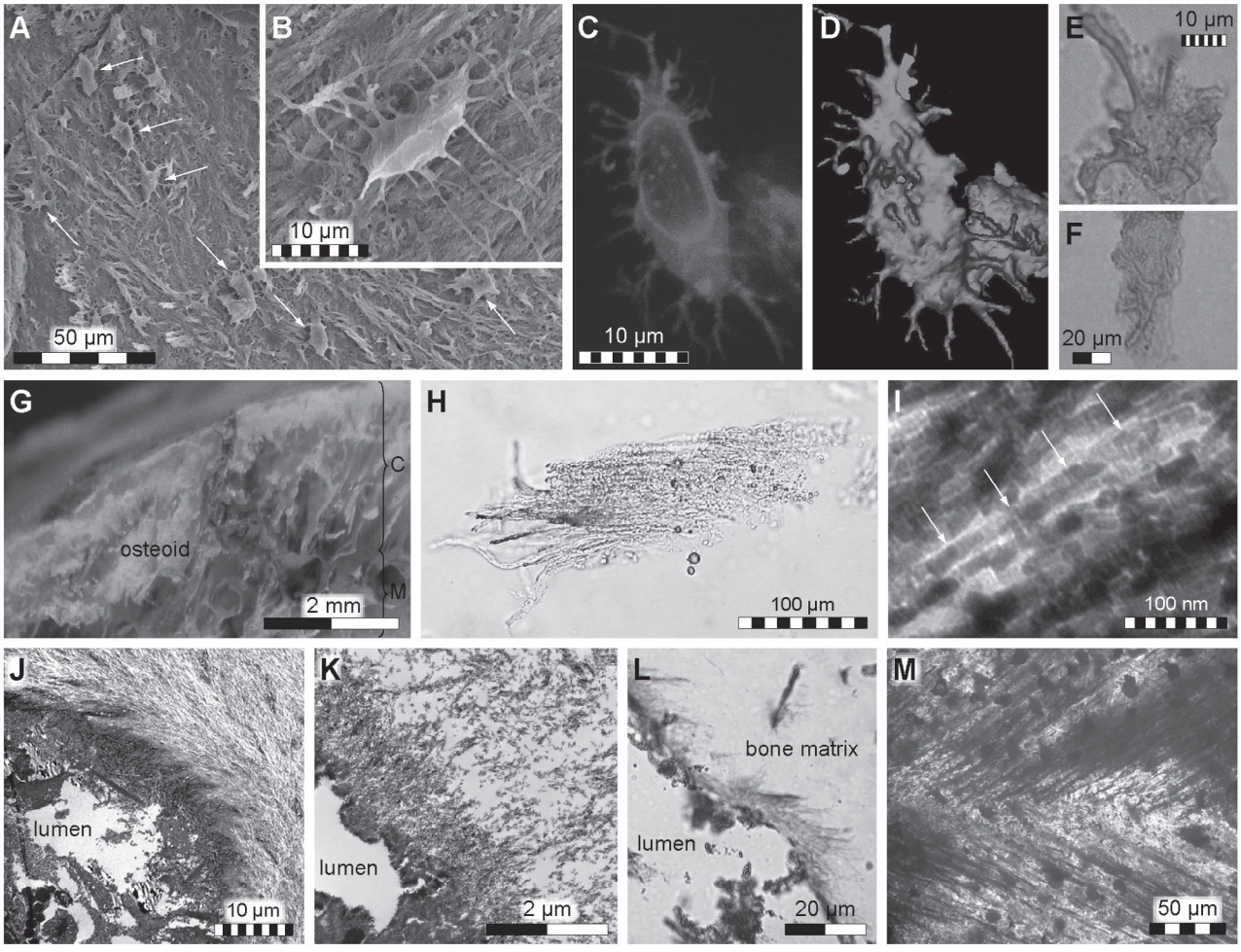

Figure 1. Figure 1 from Lindgren,[20] shows still-bright (i.e. not darkened by the Toast model's AGE's) Cretaceous mosasaur soft tissues. In particular, E, F) Light micrograph of likely osteocytes. G) Light micrograph of whitish, demineralised osteoid tissue showing cortex (c) and medulla (m). H) Light micrograph of an isolated fiber bundle. L) Light micrograph of histochemically stained (blue) connective tissue. M) Light micrograph of untreated thinsection shows fibers embedded in hydroxyapatite.

polymers in general as "reinforced AGE/ALE scaffolds". Yet, this form of reinforcement should cause stiffening of the tissue. For example, tissue specimens that retained some of the morphology and chemistry of blood vessels and nerves in decalcified Jurassic paleonisciform scales were "brittle and cracked".[1]

However, some published observations are difficult to reconcile with the reduction in flexibility inherent to peptide polymerisation. For example, the still relatively bright *Tyrannosaurus* tissue was previously described by Schweitzer *et al.* as "flexible vascular tissue that demonstrated great elasticity and resilience upon manipulation".[5] Plus, blood vessels extracted from a Brachylophosaurus' femur were "still soft, hollow structures".[8]

Similarly, there does not appear to be even a hint of stiffening in a report of Ediacaran *Sabellidites* fossils:

> "Minerals have not replicated any part of the soft tissue and the carbonaceous material of the wall is primary, preserving the original layering of the wall, its texture, and fabrics."[9]

This study described the worm sheath as still "flexible, as shown by its soft deformation".[9] As noted, such flexibility is not consistent with the claims regarding the polymer shield model.

The polymer shield concept also clashes with immunological results. If AGEs shield proteinaceous material from microbes and water, they should also shield them from antibodies. However, neither molecular nor mineral shielding appears to have hindered antibodies from binding directly to dinosaur actin, tubulin, and PHEX.[10]

What is more, AGE formation results in changing amino acids into more stable aromatic heterocycles. However, this chemical alteration would interfere with identifying specific amino acid sequences, which is inconsistent with reports of specific dinosaur protein sequences.[11–13] Apparently, these particular protein fragments supposedly survived far longer than biochemical predictions without exposure to (and thus protection from) AGE-forming chemistry.

Finally, other reports describe original organics within endogenous soft tissues such as skin and visceral or cranial organs.[14–16] Pliable tissue remnants found outside the originally hard tissues described by Wiemann *et al.* call for alternate or amended preservation modes. Although the set of fossils included by Wiemann and her colleagues show evidence of cross-linked peptide polymer shields, other published results including those noted above that describe white or transparent, flexible tissues show no association with AGE's and thus do not conform to the shield model. Therefore, this report should instead have stated: "This *may* explain *some modest* preservation of fragile soft tissues in certain chemical environments … ."

A longevity experiment would address conflicting evidence

If polymer shields are real, can they last millions of years? Longevity experiments would add empirical support to the claim that N-heterocylic polymers shield nearby proteins through deep time. The colour differences and Raman spectra presented in Wiemann and coworkers may support the presence of N-heterocyclic polymers, and they may help explain the preservation of some fragile tissues, especially within the biblical time frame of thousands of years. However, neither colour change nor Raman spectra substitute for longevity experiments that would provide direct support for polymer shield persistence through deep time. The authors appear to accept for a circular argument in place of an empirical determination of N-heterocyclic polymer longevity. Though not explicitly stated, they imply that because fossil tissue presumably has survived millions of years, then obviously the N-heterocyclic polymers within the fossil must also have survived through deep time. Experimental decay results could increase confidence in the accuracy of this aspect of their conclusion and could address several inconsistencies.

Firstly, data on the decay of synthetic polymers can help in understanding decay of fossil polymers. Plastics that are specifically designed to resist chemical decay and microbial degradation are thicker and more robust than AGEs, and likely comprised of higher molecular weights than polymers formed from fossilisation. Yet even the most recalcitrant synthetic polymers can begin to break down within a human lifespan. "There are different types of polymer degradation such as photo-, thermal-, mechanical and chemical degradation."[17]

In addition, there remains a need for direct evidence to support the assertion that AGEs resist microbes more than any unaltered proteinaceous material. The ubiquity of microbes, their known capacity to degrade all major classes of polymers, and their tendency to degrade polymers of biological origin more readily than synthetic polymers challenge the idea.[18] Decay studies of AGEs/ALEs are therefore necessary to substantiate the claims made by Wiemann and her coworkers and to explain why fragile organic polymers should be expected to outlast robust synthetic polymers.

Bone collagen decay rates are well characterised,[19] but it remains unclear how fast N-heterocyclic polymers decay. Since collagen is already known to be insoluble and slow-to-decay, it may well outlast AGEs. Without knowing either the proximity of these two organic components to one another or their respective decay rates, claims that N-heterocyclic polymers protect proteins are premature.

Conclusion

The N-heterocyclic polymer shield concept offered by Wiemann and co-workers has strengths and weaknesses. It does not explain the light colour, flexible texture, or immunological stain patterns of certain published soft tissue fossils. For this reason, it cannot be invoked to explain all soft tissue fossils, but only those that show evidence of AGEs. Also, decay features of synthetic polymers indicate that more work is required to justify the claim that diagenetic polymers persist through deep time, let alone the claim that they can shield nearby proteins for that long. The conclusion of Wiemann *et al.* went beyond their data and required longevity studies to justify it. Despite these important distinctions, introducing N-heterocyclic polymers in early fossilisation contributes to the ongoing and challenging task of explaining soft tissue preservation over even thousands of years. The presence of these secondary polymers in fossils is bolstered by both darkening effects and Raman spectral evidence described by Wiemann *et al.*, but further research is needed to gauge their longevity and effectiveness in shielding nearby proteins.

References

1. Wiemann, J. *et al.*, Fossilization transforms vertebrate hard tissue proteins into n-heterocyclic polymers, *Nature Communications* **9**:4741, 2018.
2. Schweitzer, M.H., Soft tissue preservation in terrestrial mesozoic vertebrates, *Annual Review of Earth and Planetary Sciences* **39**:187–216, 2011.
3. Surmik, D. *et al.*, Spectroscopic studies on organic matter from triassic reptile bones, upper silesia, poland, *PLoS One* **11**:e0151143, 2016 | doi:10.1371/journal.pone.0151143.
4. Lindgren, J. *et al.*, Soft-tissue evidence for homeothermy and crypsis in a jurassic ichthyosaur, *Nature* **564**:359, 2018.
5. Schweitzer, M.H. *et al.*, Soft-tissue vessels and cellular preservation in *Tyrannosaurus rex*, *Science* **307**:1952–1955, 2005 | doi:10.1126/science.1108397.
6. Schweitzer, M.H., Wittmeyer, J. L. and Horner, J. R., Soft tissue and cellular preservation in vertebrate skeletal elements from the Cretaceous to the present, *Proc. Biol. Sci.* **274**:183–197, 2007 | doi:10.1098/rspb.2006.3705.
7. Ciulla, M. *et al.*, Fibrosis, enzymatic and non-enzymatic cross-links in hypertensive heart disease, *Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders)* **11**:61–73, 2011.
8. Cleland, T.P. *et al.*, Mass spectrometry and antibody-based characterization of blood vessels from *Brachylophosaurus canadensis*, *J. Proteome Res.* **14**:5252–5262, 2015 | doi:10.1021/acs.jproteome.5b00675.
9. Moczydłowska, M., Microstructure and biogeochemistry of the organically preserved ediacaran metazoan sabellidites, *J. Paleontology* **88**:224–239, 2014.
10. Schweitzer, M.H. *et al.*, Molecular analyses of dinosaur osteocytes support the presence of endogenous molecules, *Bone* **52**:414–423, 2013 | doi:10.1016/j.bone.2012.10.010.
11. Asara, J.M. *et al.*, Protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry, *Science* **316**:280–285, 2007 | doi:10.1126/science.1137614.
12. Schweitzer, M.H. *et al.*, Biomolecular characterization and protein sequences of the campanian hadrosaur *B. canadensis*, *Science* **324**:626–631, 2009 | doi:10.1126/science.1165069.
13. Schroeter, E.R. *et al.*, Expansion for the *Brachylophosaurus canadensis* Collagen I sequence and additional evidence of the preservation of Cretaceous protein, *J. Proteome Res.* **16**:920–932, 2017 | doi:10.1021/acs.jproteome.6b00873.
14. Lindgren, J. *et al.*, Convergent evolution in aquatic tetrapods: insights from an exceptional fossil mosasaur, *PLoS One* **5**:e11998, 2010 | doi:10.1371/journal.pone.0011998.
15. Lindgren, J. *et al.*, Soft-tissue evidence for homeothermy and crypsis in a jurassic ichthyosaur, *Nature*, 2018 | doi:10.1038/s41586-018-0775-x.
16. Edwards, N.P. *et al.*, Infrared mapping resolves soft tissue preservation in 50 million year-old reptile skin, *Proc. Biol. Sci.* **278**:3209–3218, 2011 | doi:10.1098/rspb.2011.0135.
17. Göpferich, A., Mechanisms of polymer degradation and erosion, *Biomaterials* **17**:103–114, 1996.
18. Gu, J.-D., Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances, *International Biodeterioration & Biodegradation* **52**:69–91, 2003.
19. Buckley, M. *et al.*, Comment on "Protein sequences from mastodon and tyrannosaurus rex revealed by mass spectrometry", *Science* **319**:33; author reply 33, 2008 | doi:10.1126/science.1147046.
20. Lindgren, J.P., Uvdal, A., Engdahl, A.H. *et al.*, Microspectroscopic evidence of cretaceous bone proteins, *PLoS One* **6**(4):e19445, 2011.