Astonishing Soft Tissue Permanence in Surface Collected Triceratops Horn Shards from Hell Creek MT, USA

Jonas Cruz, Mark H Armitage

Microscopy and Microanalysis, **30** (Suppl 1), 2024, 2020–2022
https://doi.org/10.1093/mam/ozae044.998

Microscopy AND Microanalysis

Meeting-report

Astonishing Soft Tissue Permanence in Surface Collected *Triceratops* Horn Shards from Hell Creek MT, USA

Jonas Cruz[1,2] and Mark H Armitage[1,*]

[1]*Dinosaur Soft Tissue Research Institute, Sequim, WA, USA*
[2]*Enlightium Academy, El Paso, TX, USA*

*Corresponding author: profmark@dstri.org

The Hell Creek formation in Montana and South Dakota, USA has famously yielded many instances of dinosaur soft tissue (dST) elements [1-3]. In most cases the detail of preserved cells, vessels and nerves liberated through decalcification is startling in light of the vast time periods involved and the associated impact of soil scavengers, decomposers, and severe environmental pressures placed upon buried remains. Until 2020, a review of the most cited reports of dST might have left the wrong impression that the osteocytes (bone cells), blood vessels and red blood cell-like structures discovered have only come from a few specimens of minimally disarticulated limb bones (mostly encapsulated femurs) that were deeply buried in fluvial sandstones, largely undisturbed over deep time and not scavenged by bacteria, fungi, microbes, worms, insects or rodents. Notable examples are *T. rex* [1-5, 7-10], *B. canadensis* [6, 8] and *H. stebingeri* [11], especially MOR 555, MOR 1125, MOR 2598 and MOR 548 all from the Museum of the Rockies [1-11].

Alternately, our work has revealed the presence of osteocytes, vessels and RBC-like structures and more, (including cells with nuclei, veins, vessels, vein valves, massive clots in bone canals) within fractured, isolated, incomplete and disarticulated specimens of skull, horns, ribs, vertebra, frill, and condyle from *Triceratops*, *Nanotyrannus* and *Dimetrodon* [12-17, 20-22]. We also reported on nerves from limb bones of Permian tetrapods *Cacops* and *Dimetrodon* [18-19, 23]. To date the present workers alone report peripheral nerve elements from dinosaur bone [17-19, 23].

Further, we have observed and collected nerve filaments from disarticulated and often partially crushed *Nanotyrannus* [21, unpublished] and *Edmontosaurus* [22, unpublished] ribs, vertebrae and scapulae. Recently we reported significant infestations of free-living, opportunistic nematodes in *Nanotyrannus* rib and vertebra, *Triceratops* condyle, frill, horn, rib and vertebra and *Edmontosaurus* scapula, jaw and rib [23]. Large nematode infestations, especially juveniles, within Hell Creek bones would suggest substantial bacterial and fungal presence. This would support the communities of nematodes observed [23]. It would follow that there is enough decaying tissue within the bones to first attract and then support robust nematode communities.

Surprising permanence and recovery of soft tissues has been reported from long-held museum specimens of dinosaur remains collected within the Lance Formation, deposited at the same time as Hell Creek [24].

Deep time preservation mechanisms for soft tissues have been proposed [1, 2, 4, 5, 7-9, 11]. Such mechanisms seem dependent on quick burial in sandy soils, minimal disarticulation, long term sequestration and minimal disturbance over time, yet we continue to observe well-preserved soft tissues in fractured, weathered and heavily predated near-surface specimens [12-23].

Near-surface bones at Hell Creek are certainly exposed to freeze-thaw cycles, meltwater and rainwater infiltration, scavenging by fungi, bacteria, microbes, worms, rodents and insects, and permineralization via calcification and silicification from saturated water infiltration. The level of preservation of dST is certainly surprising and controversial.

Methods

In May of 2023, we returned to the collection site of the *Triceratops* supra-orbital horn reported by Armitage and Anderson in 2012 [13-16]. We examined the surface area at the site from which the *Triceratops* horn was collected. We salvaged bone shards still present at the surface. Shards were placed in 10% formalin at the site, were washed and demineralized, and were air-dried. Our goal was to examine these unburied shards for soft tissues via demineralization and thin sectioning.

Results

Post-decalcification solutions yielded soft osteocytes (Figures 1a, b, c, d), vessels (Figure 2), and peripheral nerve filaments (Figures 3a,b,c). Osteocyte cells filled lacunae in thin sections of bone and featured robust filipodia extending into canalicular spaces (Figure 1a). Whorls of cells were also present (Figure 1a). Shards which were demineralized over time, yielded cells and nerves at regular collection intervals some very well preserved. These well-preserved tissue elements are typical for all *Triceratops* remains collected at Hell Creek, often those from deeper sediments (Figures 1-3, 5,6). Large numbers of individual cells were collected from the bottoms of dishes from these surface shards (Figure 1d). Nerves were also readily evident and were severely dehydrated in some instances. Nerve filaments were always birefringent under crossed polars and easily seen in oblique lighting. Most exhibited 'scalloped' and contracted edges which pronounce the appearance of desiccation atrophy along the filament. In some specimens, collagen wrappings of the *epineurium* and *perineurium* layers were separated enough by dark lined gaps occurring between them to visualize (Figure 3c). This revealed the grossly contracted and somewhat twisted features of the outer collagen layer in desiccated filaments, yet the fine details of deeper wrappings were clearly visible in fibers that appeared turgid, wet and clear (Figures 3a, b, c). One reason the nerves may have exhibited this separation of collagen in the wrappings might be a result from our immersing of nerves into room temperature 4% aqueous solutions of osmium tetroxide for at least a month after

collection. Similar extant nerves turned darker to black immediately but these Cretaceous nerves only darkened somewhat. The finely arranged criss-crossed fibers of the wrappings seen in Figures 3a, b, and c are typical of the best well-preserved Cretaceous *Triceratops* nerves from Hell Creek.

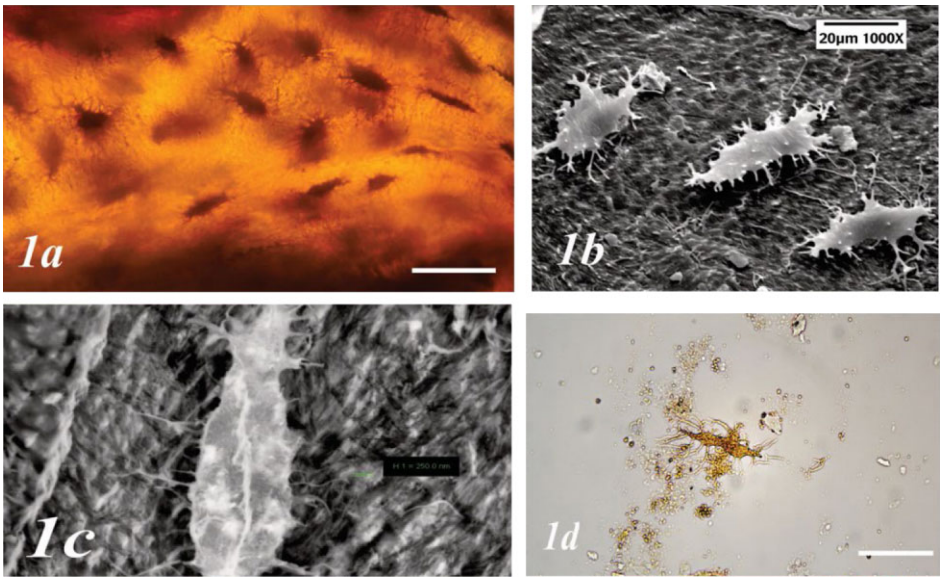

Figure 1. (a) Light micrograph, 40um thin section of *Triceratops* **surface shard**, whorl of osteocytes still present in bone. Note robust cells with filipodia. Scale bar = 24um. (b) Scanning electron micrograph (SEM) of *Triceratops* **surface shard** cells uncovered by partial demineralization. Coated specimen. Scale bar = 20um. These cells are typical and common in all *Triceratops* shards and fractured bones studied. (c) SEM of single osteocyte from *Triceratops*, uncoated **buried shard**, Hitachi variable pressure SEM. These cells are typical and common in all *Triceratops* shards from Hell Creek. Scale bar = 200nm between green lines. (d) Light micrograph, EDTA-liberated osteocyte from *Triceratops* **surface shard**. Scale bar = 12um.

Figure 2. Light micrograph, blood vessel from Triceratops **surface shard**. Scale bar = 30um.

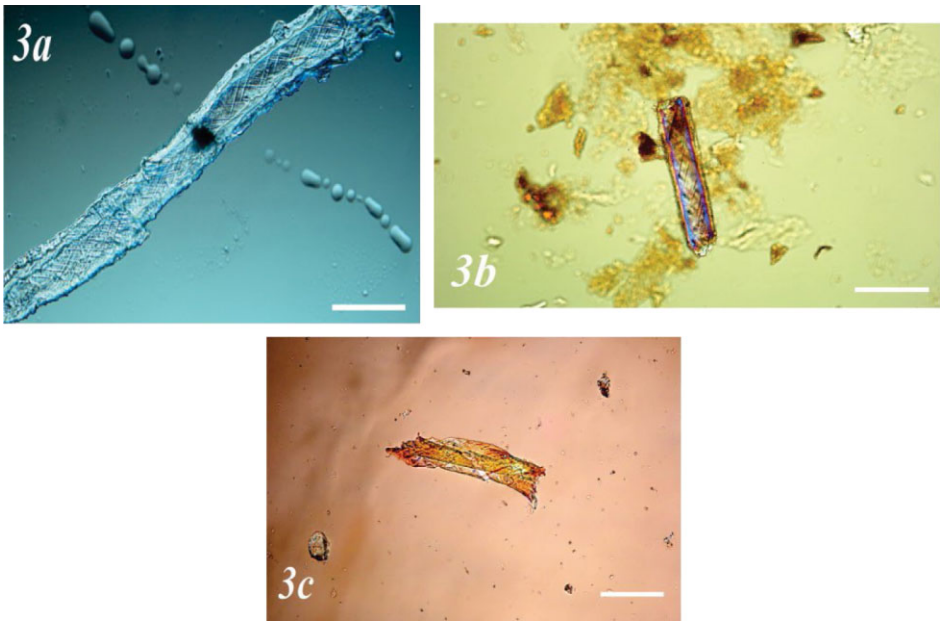

Figure 3. (a) Light micrograph, DIC, liberated nerve filament, *Triceratops* **buried shard**. Note lipid droplets across field. These nerve filaments are typical and common in *Triceratops* shards and fractured bones studied. Scale bar = 80um. (b) Polarized light micrograph, liberated nerve filament, *Triceratops* **surface shard**. These nerve filaments are typical and common in *Triceratops* shards and fractured bones studied. Scale bar = 80um. (c) Polarized light micrograph, liberated nerve fragment *Triceratops* **surface shard**. Note outer epineurium cover Scale bar = 50um.

References

1. MH Schweitzer and JR Horner, JR, *Annales de Paléontologie* **85** (1999), p. 179–192. https://www.researchgate.net/search.Search.html?query=intravascular+microstructures&type=publication.
2. MH Schweitzer *et al.*, *Science* **307** (2005), p.1952–55. DOI:https://doi.org/10.1126/science.1108397.
3. MH Schweitzer, *et al.*, *Science* **308** (2005), p.1456-1460. DOI: https://doi.org/10.1126/science.1112158.
4. MH Schweitzer *et al.*, *Proceedings Royal Society B* 274 (2007), p.183–97. DOI: https://doi.org/10.1098/rspb.2006.3705.
5. MH Schweitzer, *et al.*, *C R Paleovol* 7 (2008), p.159-184.DOI: https://doi.org/10.1016/j.crpv.2008.02.005.
6. MH Schweitzer *et al.*, *Science* **324** (2009), p.626–31. DOI: https://doi.org/10.1126/science.1165069.
7. MH Schweitzer *et al.*, *Bone* **52**(1) (2013), p.414–23. DOI: https://doi.org/10.1016/j.bone.2012.10.010.
8. MH Schweitzer, *et al.*, *Proceedings of the Royal Society B* **281**: 2013274. DOI: http://dx.doi.org/10.1098/rspb.2013.2741.
9. EM Boatman, *et al.*, *Scientific Reports* (**9**)15678, (2019) DOI: https://doi.org/10.1038/s41598-019-51680-1.
10. AM Bailleul *et al.*, *PeerJ* **7**:e7764 DOI: https://doi.org/10.7717/peerj.7764.
11. AM Bailleul *et al.*, *National Science Review* **7** (2020), p.815–22. DOI: https://doi.org/10.1093/nsr/nwz206.
12. MH Armitage, *American Laboratory* **44**(10) (2012), Cover.
13. MH Armitage and KL Anderson, *Acta Histochemica* **115** (2013), p.603-608. DOI: http://dx.doi.org/10.1016/j.acthis.2013.01.001.
14. MH Armitage and KL Anderson, *Microscopy and Microanalysis* **20** (S3) (2014), p. 1274-1275. DOI: https://doi.org/10.1017/S1431927614008101.
15. MH Armitage, *Creation Research Society Quarterly* **51**(4): 248-257.
16. MH Armitage, *Microscopy Today* **24**(1) (2016), p.18-23. DOI: https://doi.org/10.1017/S1551929515001133.
17. MH Armitage and J Solliday, *Microscopy Today* **28**(5) (2020), p.30-38. DOI: https://doi.org/10.1017/S1551929520001340.
18. MH Armitage, *Microscopy Today* **29**(2) (2021), p.20-25. DOI: https://doi.org/10.1017/S1551929521000468.
19. MH Armitage, *Microscopy Today* **31**(1) (2023), p.32-35. DOI: https://doi.org/10.1093/mictod/qaac005.
20. MH Armitage *Microscopy Today* **30**(1) (2022), p.18-23. DOI: https://doi.org/10.1017/S1551929521001565.
21. MH Armitage, *Microscopy Today* **30**(6) (2022), p.34-39. DOI: https://doi.org/10.1017/S1551929522001262.
22. MH Armitage, *Journal of Creation* **37**(3) (2023), p.55-62. https://dstri.org/wp-content/uploads/2023/11/8_extracted_2023vol37-3JOC.pdf.
23. MH Armitage, *Microscopy Today* **32**(1) 2024), p.26-34. DOI: https://doi.org/10.1093/mictod/qaad110.
24. S Bertazzo *et al.*, *Nature Communications* **6**(7352) (2015), DOI: https://doi.org/10.1038/ncomms8352/10.1038/ncomms8352.