Bone Canal Clots in Surface Collected Shards of Triceratops Horn are Disrupted by Exposure to Surface Environment Conditions in MT, USA

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Bone Canal Clots in Surface Collected Shards of *Triceratops* Horn are Disrupted by Exposure to Surface Environment Conditions in MT, USA

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The findings of soft tissue in dinosaur bones remain controversial, yet reports of soft tissue continue to be made [1, 2]. To date, approximately 140 individual dinosaur bones have been demineralized (and reported) specifically for the purpose of collecting and characterizing soft tissues that might remain within them [1]. Shards are often collected along with complete dinosaur bones for analysis, however single shards do not appear to be widely studied histologically or via demineralization [1, 2].

In 2023, we returned to the original site where a complete, isolated and fractured *Triceratops* horn was recovered at Hell Creek (Glendive, MT, 2012) [3]. We examined the surface at the site and a small, narrow gully channel directly proximal to the collection area and found several shards of bone, associated with the horn removal, which had been left behind. These appeared to have been dislodged from the soil over time and some made their way down along the channel either by wind or rain action. Shards were immersed into 10% formalin at the site and were transferred to the laboratory for decalcification and thin sectioning. Fixation extended for 3 weeks and bones were water washed and air-dried. Demineralization of shards yielded an abundance of exceptionally preserved osteocytes and nerves, and is reported elsewhere [4]. The purpose of this study was to examine thin sections of surface collected bone shards for the presence of blood clots in vessel canals using UV autofluorescence microscopy.

Multiple shards of varying-sized buried bone from Hell Creek have demonstrated robust iron-rich clots adhering tightly to blood canal walls throughout [3, 5-8]. Clots have been found in *Triceratops* horn, [3], *Triceratops* vertebra, rib and frill [5], and *Dimetrodon* femur, rib and jaw [6]. Clots were also reported in *Nanotyrannus* radius, limb elements, digit elements, vertebra, ulna, and claw [7]. *Edmontosaurus* rib and scapula, *Camarasaurus* limb, *Cacops* humerus, *Eryops* humerus, *Varanops* femur and *Captorhinus* humerus have all presented positive for iron-rich canal clots [8].

Surface shards studied here were angular with sharp corners along fracture margins, indicating these had not rolled across the surface [9]. Rolling or at the least, skidding would be expected, given normal wind, rain and snow events in Montana and given the gully channel present. Cattle and other quadrupeds roam the Baisch Ranch in MT and could have disturbed bones as well. Other workers have documented examples of atrophy in surface specimens resulting from such exposure [9]. All shards in this study were much darker in color (almost black in some instances) than were buried bones from the same site, possibly as a result of dehydration and exposure to the surface. Several of the shards exhibited significant lichen growth on the top facing surfaces of shards (not shown).

Thin sectioning revealed robust blood clots reacting to UV light within most bone canals (Figures 1a, 1b). Grossly clotted vessels were evident to the naked eye in hand-sized pieces of unidentified bone lying on the ground (Figure 1c). After demineralization and sectioning of similar bone at the same site, vessels filled with clots displayed the crystallized nature of the blood products, which remained in canals (Figures 1d, 1e). Mineral infill from the surface was evident in many bone canal sections (not shown), particularly canals associated with the edges of the shards (Figure 1b). Due to water infiltration at the surface, minerals entered canals and the resulting infill abutted against and crumbled clots partially (Figure 1b), dislodging much clot material from bone canals. Spaces where clots were pushed out were occupied by infill characterized by individual, large and small angular grains. Individual mineral grains auto-fluoresced brightly in green and orange color within infilled canals (not shown). Many clots appeared atrophied, (i.e., not as bright in UVFL and appearing somewhat diffuse and frayed) in canals where part of the clots were disturbed or missing (Figure 1b). Even when portions of clots were missing, the remaining material still clung tenaciously to canal walls and auto-fluoresced brightly. SEM analysis of other partly-buried shards from the same site revealed the tight junction between the clots and bone canal walls (Figure 1e).

Whole shard elemental (EDS) analysis shows the highest concentrations of iron (Figure 1f, red), and oxygen (Figure 1g, green), are found in the clots and not distributed throughout the bone matrix. Iron is associated mostly with the clots and not with bone itself, where osteocytes are populated. This may be an impediment to the iron-mediated radical formation theory of deep time 'cross-linking.' This scenario is dependent on the persistence of water and iron within bone matrix for osteocytes to be 'cross-linked' [2] and thus preserved, yet we see little iron in the bone matrix in a specimen that has yielded astonishingly preserved osteocytes and nerves [4].

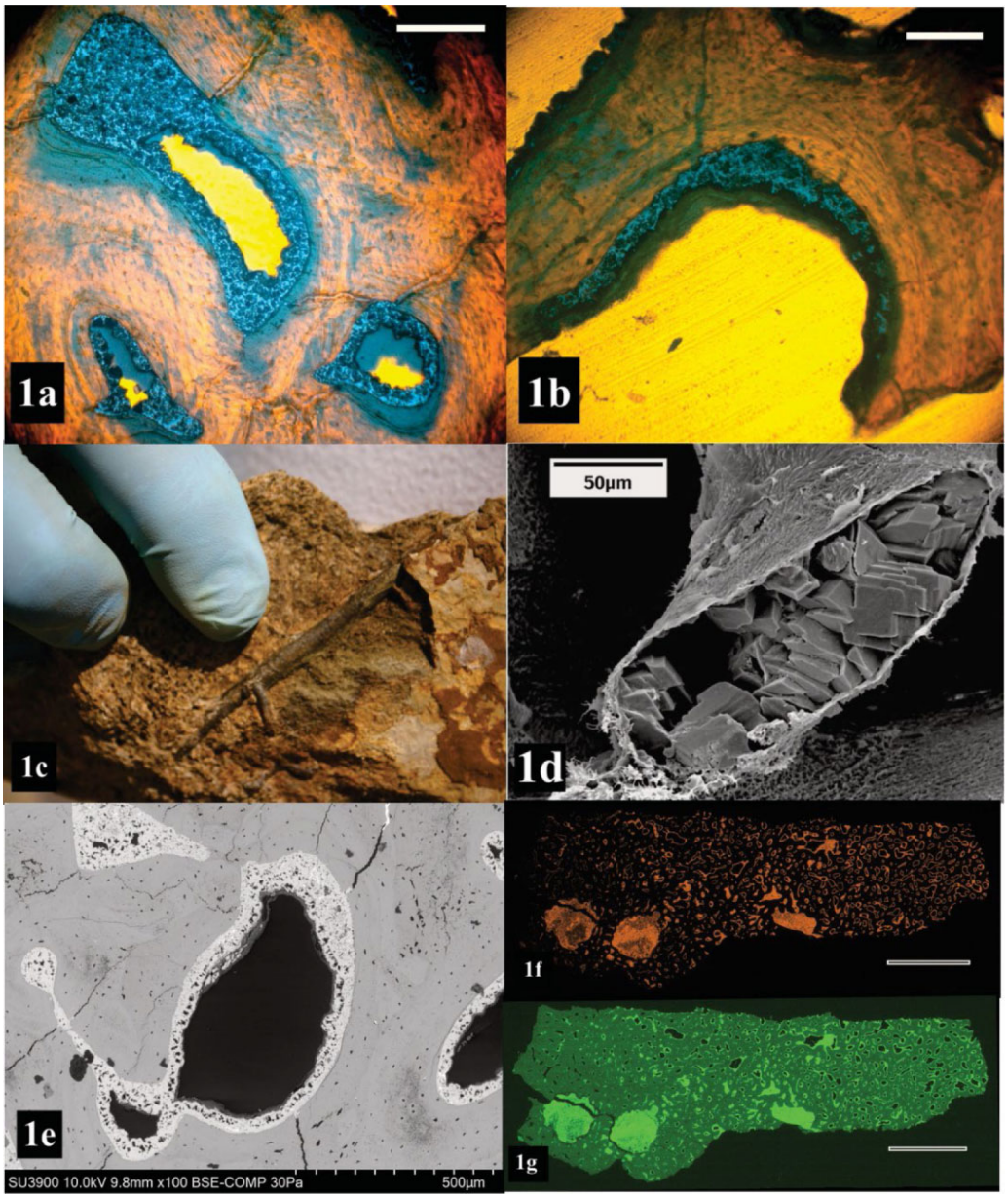

Figure 1. a) Thin section, surface collected *Triceratops* horn bone shard in transmitted light with reflected light UVFL. Note autofluorescence of iron in clot. Scale bar =300 um. b) Thin section, surface collected *Triceratops* horn bone shard in transmitted light with reflected light UVFL. Note autofluorescence of iron in clot. Scale bar =150 um. c) Gross clot embedded in surface collected unidentified *Triceratops* bone. d) SEM, blood vessel in partially demineralized, partially buried *Triceratops* horn shard from the same site. Note internal crystallized blood clot. Scale bar = 50um. e) SEM of thin section from partially buried *Triceratops* horn shard from the same site, (no cover glass, no coating). Dotted scale bar = 500um. Note tight junction between clot and bone canal wall. f) EDS elemental analysis of surface-collected bone shard, Iron distribution (red) is restricted mostly to clots. Scale bar = 5mm. g) EDS elemental analysis of surface-collected bone shard, Oxygen distribution (green) is restricted mostly to clots. Scale bar = 5mm.

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