Testing microstructural adaptation in the earliest dental tools

David Jones[1,*], **Alistair R. Evans**[2],
Emily J. Rayfield[1], **Karen K. W. Siu**[3,4]
and Philip C. J. Donoghue[1]

[1]*School of Earth Sciences, University of Bristol, Wills Memorial Building, Queen's Road, Bristol, BS8 1RJ UK*
[2]*School of Biological Sciences, and* [3]*Monash Biomedical Imaging, School of Physics, Monash University, Clayton, Victoria 3800, Australia*
[4]*Australian Synchrotron, 800 Blackburn Road, Clayton, Victoria 3800, Australia*
*Author for correspondence (david.jones@bris.ac.uk).

Conodont elements are the earliest vertebrate dental structures. The dental tools on elements responsible for food fracture—cusps and denticles—are usually composed of lamellar crown tissue (a putative enamel homologue) and the enigmatic tissue known as 'white matter'. White matter is unique to conodonts and has been hypothesized to be a functional adaptation for the use of elements as teeth. We test this quantitatively using finite-element analysis. Our results indicate that white matter allowed cusps and denticles to withstand greater tensile stresses than do cusps comprised solely of lamellar crown tissue. Microstructural variation is demonstrably associated with dietary and loading differences in teeth, so secondary loss of white matter through conodont phylogeny may reflect changes in diet and element occlusal kinematics. The presence, development and distribution of white matter could thus provide constraints on function in the first vertebrate dental structures.

Keywords: finite-element analysis; conodont; white matter; teeth

1. INTRODUCTION

Teeth exhibit functionally significant microstructural variation, demonstrably associated with dietary and occlusal differences [1]. Even greater microstructural variation is present in conodont elements, the earliest vertebrate dental structures [2]. The microstructure of conodont lamellar apatite, a functional analogue and putative biological homologue of enamel, from which element crowns are predominantly constructed [3] was also adapted to specific occlusal kinematics [4]. However, in many conodont taxa, the dental tools responsible for food acquisition and fracture (cusps and denticles) are composed partially or entirely from an additional tissue known as 'white matter' [5] (figure 1*a*,*b*).

The homology, structure and ontogeny of white matter are contentious and its interpretation has been central to debate over conodont skeletal tissue homology and the phylogenetic position of conodonts. Although it has been considered a secondary, perhaps

diagenetic, state of lamellar crown tissue [6,7], white matter is now widely interpreted as a primary tissue that developed in association with lamellar crown tissue [8]. Initially interpreted as cellular dermal bone [8], it has subsequently been compared with mesodentine [3,9], enameloid [2,10] and interpreted as a conodont-specific tissue [11]. It was originally considered to comprise a microcrystalline aggregate [3], although more recently has been reinterpreted to be composed of a small number of larger crystals [12].

Although it often intergrades with lamellar tissue, white matter is a conodont subclade autapomorphy [2,3,11]; no comparable tissue is found in any other animal group. It was evolutionarily labile, repeatedly lost or reduced in many lineages [13]. Several workers have suggested that, like variation in lamellar tissue, the evolution of white matter was a functional adaptation that could have provided a structural benefit, reducing brittle failure by providing decussation planes (at its interface with lamellar tissue) [3] and internal vacuities to control crack propagation; but the structural arrangement of material properties in white matter may also have offered advantages in reducing stress within conodont dental tools. We test this hypothesis quantitatively, using finite-element (FE) modelling, an engineering technique that permits virtual experimentation upon structures too small and delicate for empirical analysis. To do this, we apply identical loading to two FE models of a conodont cusp—one simulating white matter, the other lamellar tissue—and compare the relative stress magnitudes.

2. MATERIAL AND METHODS

We focused on the P_1 elements of the conodont skeleton, paired food processing structures at the posterior of the feeding apparatus, because their occlusal kinematics are best understood [14]. We analysed *Wurmiella excavata*, because it is represented by well-preserved material and its P_1 elements form denticulated blades, a morphology common to many conodont species. We selected the cusp of the sinistral element of a pair of articulated *W. excavata* P_1 elements as a representative geometry (Royal Ontario Museum 61381), since the cusps and denticles are morphologically similar in this taxon. We acquired high-resolution (voxel size: 0.5 μm) three-dimensional data from synchrotron radiation X-ray computed micro-tomography (SRμCT) at the SPring-8 lightsource, Japan. The specimen was scanned on the 20XU beamline, collecting 1800 projections at an energy of 24.65 keV.

Surfaces were extracted from volume data using AVIZO v. 6 (VSG) and imported into ABAQUS v. 6.7 (Simulia), where FE models were created and analysed. An isotropic model simulated a cusp composed of white matter. For comparison, we used an orthotropic model to simulate a cusp constructed of lamellar tissue where apatite crystallites are orientated orthogonal to the surface, a common microstructure for this tissue [4]. To model these properties, the orthotropic model was partitioned into 16 longitudinal segments (figure 1), and the stiffest axis in each segment orientated approximately perpendicular to the model surface, forming a radiating configuration around the long axis of the model. The isotropic model was identically partitioned (without stiffness variation) for comparability. Like enamel, both lamellar tissue and white matter were composed of hypermineralized hydroxyapatite [15], so we use values from FE and empirical analysis of extant enamel to furnish reasonable estimates of element material properties [16,17]. We assign the stiffest axis of our orthotropic model an elastic modulus of 75 GPa, and the remaining two axes 40 GPa. The cancellous white matter is less dense and thus less stiff; we tried several stiffness values (see §3). For both models, we assume homogeneity, a Poisson ratio of 0.23 and a shear modulus of 30 GPa [18,19]. Models were constrained at the base in 6 d.f. and meshed with 75 746 C3D4 4-node linear tetrahedral elements.

Uniform pressure was applied in two loading directions to each model: to the tip, simulating initial puncture cut into food, and to the dorsal edge, simulating movement through the food in a draw cut. Puncture cutting involves movement parallel to the cusp long
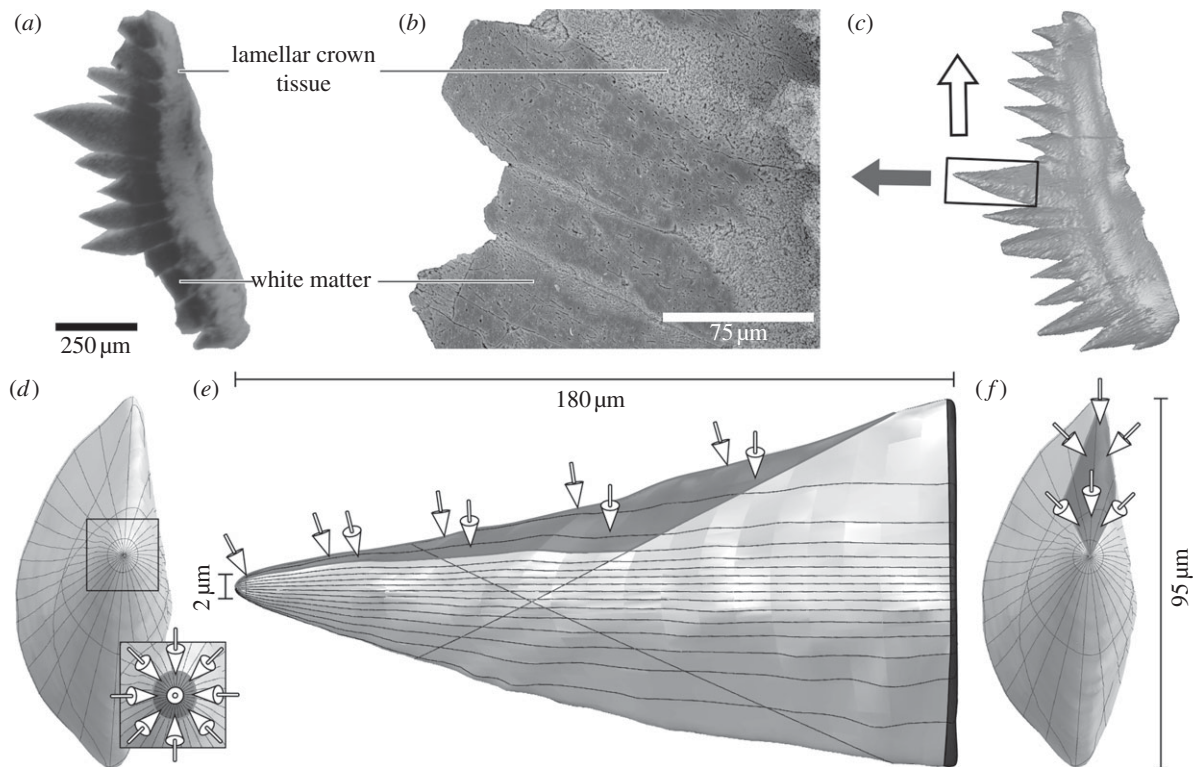
Figure 1. (*a*) *Wurmiella excavata* P$_1$ element (Silurian, Tramway Netherton, UK) in transmitted light, illustrating white matter and lamellar tissue. White matter appears darker because its interstices scatter light. (*b*) Scanning electron micrograph of *Ozarkodina confluens* S element longitudinal section showing white matter and lamellar tissue. (*c*) *Wurmiella excavata* P$_1$ element (ROM 61381) in anterior view, highlighting cusp in boxed area; arrows indicate direction of cusp motion during draw (white arrow) and puncture (grey arrow) cuts. (*d–f*) Model geometry and loading for FE analysis of *W. excavata* P$_1$ element cusp. Dark grey regions delineate area of applied load and arrows indicate its approximate direction. (*d*) Occlusal view, showing pressure applied to tip. (*e,f*) Anterior and occlusal views, showing pressure applied to dorsal edge. Black area in (*e*) indicates basal constraints for both loading conditions. Lines parallel to model long axis delineate segmentation for radial orthotropy (see text for explanation).

axis, draw cutting involves movement orthogonal to it (figure 1*c*). Force vectors are based on an occlusal model for *W. excavata* [14]. All applied pressures had a value of 10 MPa, but the absolute value is unimportant because we are interested only in comparing the difference in performance between white matter and lamellar models. Tensile stress is a good measure of fracture in mammalian enamel [20], therefore mean principal stresses were recorded and visualized for each model. These were calculated as the sum of maximum principal tensile stresses from all elements, divided by the number of elements [21]. Figure 1*d–f* illustrates model geometry, loadings and constraints.

3. RESULTS

We conducted three FE analyses for our white matter model, each using a different stiffness value: 70, 60 and 50 GPa. Mean maximum principal stress values for these moduli did not differ significantly, so only 60 GPa results are presented for comparison with the lamellar tissue model. Under each loading condition, stress patterns in models with different material properties were similar (figure 2). During puncture cutting, the white matter cusp experiences compression at the tip, whereas the lamellar model displays tensile stress. In draw cutting, both models exhibit tensile stress dorsally, which tends to increase in extent and degree towards the cusp base, but is greater in the lamellar model. The white matter model experiences more compressive stress around the ventral base of the cusp. The high stresses immediately adjacent to the base are likely to be artefacts

resulting from proximity to the constraints. Under both loading conditions, mean maximum principal tensile stresses in models simulating white matter properties are less than half of those in models simulating lamellar tissue (table 1).

4. DISCUSSION AND CONCLUSIONS

When stiffness was varied in the white matter model, tensile stresses remained similar. This stands in contrast to the difference in tensile stresses between the white matter model and the lamellar model, incorporating a similar range of stiffnesses, but in a different configuration. These results suggest that the structural arrangement of material properties in white matter allowed cusps and denticles composed of this tissue to accommodate larger loads before failing. White matter may have provided further structural advantages. Although our model deforms elastically, the high mineral to organic ratio of conodont apatite would cause it to undergo brittle failure. When cusps and denticles composed of white matter do fail completely, breaks tend to be clean and transverse, which may aid cusp or denticle re-growth [7,22]. In many taxa, white matter develops as a core within the cusp or denticle, surrounded by lamellar tissue. The interface between the two materials of differing properties may function as a decussation plane [3]. These
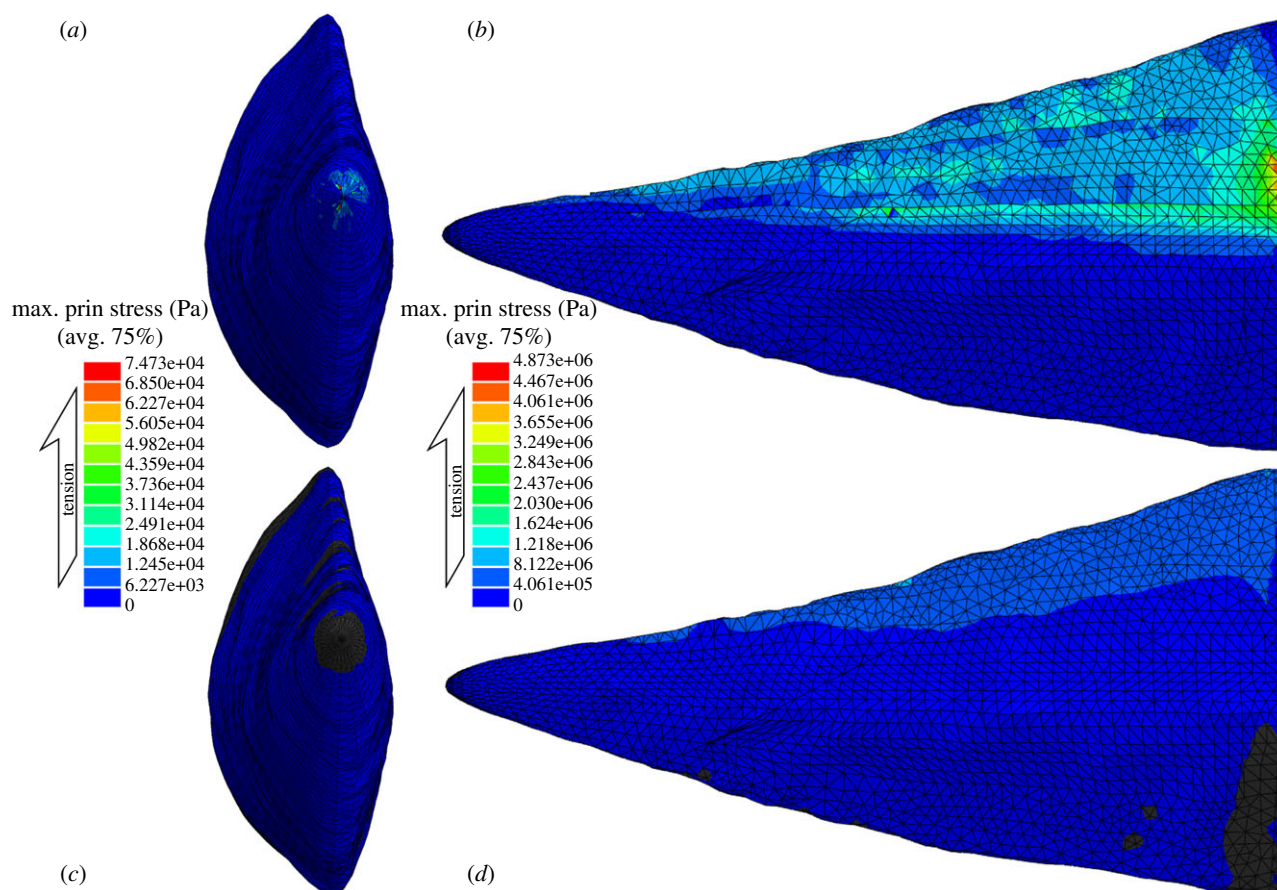
Figure 2. Maximum principal stress patterns in FE models of *W. excavata* P$_1$ element cusp: (*a*) lamellar and (*c*) white matter model under uniform tip pressure; (*b*) lamellar and (*d*) white matter model under uniform dorsal edge pressure. Models under the same loading conditions set to the same scale. Black areas indicate negative values (compression).

Table 1. Mean maximum principal stress in the *W. excavata* cusp model for the four FE analyses, with a stiffness of 60 GPa for the white matter model.

histology	kinematics	loading	mean max. principal stress (Pa)
white matter	puncture cut	tip	88
lamellar tissue	puncture cut	tip	251
white matter	draw cut	edge	144 649
lamellar tissue	draw cut	edge	301 174

planes are also formed in mammalian teeth by enamel prisms of different orientations. They combat the increasing likelihood of fracture with larger dental loads by directing cracks in suboptimal directions [1]. The interstices of white matter would increase crack tip radius and, thus, the energy required for crack propagation. Our results therefore support the hypothesis that the evolution of white matter was an adaptive response to stress resulting from food acquisition, fracture and fragmentation.

The repeated loss and reduction of this tissue through conodont phylogeny may reflect changes in the function of elements and, consequently, the diet of conodonts. White matter appears to have been lost most commonly in the prioniodinin clade [13].

Elongated cusps and denticles lacking bladed edges (i.e. round in cross section) are also prioniodinin synapomorphies [13], a morphology that is poorly adapted to fracturing viscoelastic foods. Reduction of white matter may therefore be associated with transitions to weaker foods that require lower forces for fracture. Enamel prism decussation is similarly correlated with diet and increased dental loading in mammals [1]. This hypothesis could be tested using the approach of occlusal reconstruction outlined in [14]. The presence, development and distribution of white matter may thus provide additional constraints on conodont diet and ecology, allowing more comprehensive reconstruction of trophic structure in ancient oceans.

1 Rensberger, J. M. 2000 Pathways to functional differentiation in mammalian enamel. In *Development, function and evolution of teeth* (eds M. F. Teaford, M. M. Smith & M. W. J. Ferguson), pp. 252–268. Cambridge, UK: Cambridge University Press.

2 Donoghue, P. C. J., Forey, P. L. & Aldridge, R. J. 2000 Conodont affinity and chordate phylogeny. *Biol. Rev.* **75**, 191–251. (doi:10.1017/s0006323199005472)

3 Donoghue, P. C. J. 1998 Growth and patterning in the conodont skeleton. *Phil. Trans. R. Soc. Lond. B* **353**, 633–666. (doi:10.1098/rstb.1998.0231)

4 Donoghue, P. C. 2001 Microstructural variation in conodont enamel is a functional adaptation. *Phil. Trans. R. Soc. Lond. B* **268**, 1691–1698. (doi:10.1098/rspb.2001.1728)

5 Sweet, W. C. 1988 *The Conodonta: morphology, taxonomy, paleoecology, and evolutionary history of a long-extinct animal phylum.* Oxford, UK: Clarendon Press.

6 Lindström, M. 1964 *Conodonts.* Amsterdam, The Netherlands: Elsevier Publishing Company.

7 Barnes, C. R., Sass, D. B. & Poplawski, M. L. S. 1973 Conodont ultrastructure: the family Panderodontidae. *R. Ont. Mus. Life Sci. Contrib.* **90**, 1–36.

8 Sansom, I. J., Smith, M. P., Armstrong, H. A. & Smith, M. M. 1992 Presence of the earliest vertebrate hard tissues in conodonts. *Science* **256**, 1308–1311. (doi:10.1126/science.1598573)

9 Janvier, P. 1995 Conodonts join the club. *Nature* **374**, 761–762. (doi:10.1038/374761a0)

10 Donoghue, P. C. J. & Aldridge, R. J. 2001 Origin of a mineralized skeleton. In *Major events in early vertebrate evolution* (ed. P. E. Ahlberg), pp. 85–105. London, UK: Taylor & Francis.

11 Donoghue, P. C. J. & Chauffe, K. M. 1998 *Conchodontus, Mitrellataxis* and *Fungulodus*: conodonts, fish or both? *Lethaia* **31**, 283–292. (doi:10.1111/j.1502-3931.1998.tb00518.x)

12 Trotter, J. A., Fitz Gerald, J. D., Kokkonen, H. & Barnes, C. R. 2007 New insights into the ultrastructure, permeability, and integrity of conodont apatite determined by transmission electron microscopy. *Lethaia* **40**, 97–110. (doi:10.1111/j.1502-3931.2007.00024.x)

13 Donoghue, P. C. J., Purnell, M. A., Aldridge, R. J. & Zhang, S. X. 2008 The interrelationships of 'complex' conodonts (Vertebrata). *J. Syst. Palaeontol.* **6**, 119–153. (doi:10.1017/s1477201907002234)

14 Jones, D., Evans, A. R., Siu, K. K. W., Rayfield, E. J. & Donoghue, P. C. J. 2012 The sharpest tools in the box? Quantitative analysis of conodont element functional morphology. *Proc. R. Soc. B* **279**, 2849–2854. (doi:10.1098/rspb.2012.0147)

15 Pietzner, H., Vahl, J., Werner, H. & Ziegler, W. 1968 Zur chemischen Zusammensetzung Mikromorphologie der Conodonten. *Paleontographica* **128**, 115–152.

16 Spears, I. R. 1997 A three-dimensional finite element model of prismatic enamel: a re-appraisal of the data on the Young's modulus of enamel. *J. Dental Res.* **76**, 1690–1697. (doi:10.1177/00220345970760101101)

17 Waters, N. E. 1980 Some physical and mechanical properties of teeth. In *The mechanical properties of biological materials* (eds J. F. V. Vincent & J. D. Currey), pp. 99–135. Cambridge, UK: Cambridge University Press.

18 Gilmore, R. S., Pollack, R. P. & Katz, J. L. 1970 Elastic properties of bovine dentine and enamel. *Arch. Oral Biol.* **15**, 787–796. (doi:10.1016/0003-9969(70)90042-7)

19 Lees, S. 1968 Specific acoustic impedance of enamel and dentin. *Arch. Oral Biol.* **13**, 1492–1500. (doi:10.1016/0003-9969(68)90030-7)

20 Lucas, P. W. 2004 *Dental functional morphology: how teeth work.* Cambridge, UK: Cambridge University Press.

21 Anderson, P. S. L., Gill, P. G. & Rayfield, E. J. 2010 Modeling the effects of cingula structure on strain patterns and potential fracture in tooth enamel. *J. Morphol.* **272**, 50–65. (doi:10.1002/jmor.10896)

22 Barnes, C. R., Sass, D. B. & Monroe, E. A. 1973 Ultrastructure of some Ordovician conodonts. In *Conodont paleozoology* (ed. F. H. T. Rhodes), pp. 1–30. Boulder, CO: Geological Society of America.