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Mineralized periodontia in extinct relatives of mammals shed light on the evolutionary history of mineral homeostasis in periodontal tissue maintenance

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Abstract

Aim: Dental ankylosis is a rare pathological condition in mammals, however, it is prevalent in their extinct relatives, the stem mammals. This study seeks to compare the mineralized state of the periodontal attachment apparatus between stem and crown mammals and discuss its implications for the evolution of non-mineralized periodontal attachment in crown mammals, including humans.

Materials and Methods: Thin sections of a fossil mammal and three stem mammals were compared to reconstruct periodontal tissue development across distantly related lineages.

Results: Comparisons revealed that the extinct relatives of mammals possessed the same periodontal tissues as those in mammals, albeit in different arrangements. The ankylotic condition in stem mammals was achieved through extensive alveolar bone deposition, which eventually contacted the root cementum, thus forming a calcified periodontal ligament.

Conclusions: Dental ankylosis was part of the normal development of the stem mammal periodontium for millions of years prior to the evolution of a permanent gomphosis in mammals. Mammals may have evolved a permanent gomphosis by delaying the processes that produced dental ankylosis in stem mammals. Pathological ankylosis may represent a reversion to the ancestral condition, which now only forms via advanced ageing and pathology.

Key words: alveolar bone; ankylosis; histology; osteogenesis; periodontal ligament

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Conflict of interest and source of funding statement

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Dental ankylosis in mammals involves the reduction or loss of the periodontal space by invasion of bone (Biederman 1956, Andersson et al. 1984, Andreasen 2012), a condition that is normally detrimental to the integrity of the tooth and can cause root resorption and eventual tooth loss (Atrizadeh et al. 1971, Xiong et al. 2013). Ankylosis of permanent teeth can also cause disruption to normal tooth occlusal relationships, because of the destruction of the periodontal ligament (Rubin et al. 1984). Most researchers agree that trauma to the periodontal ligament is the principal cause of ankylosis (Biederman 1956, Atrizadeh et al. 1971, Andersson et al. 1984, Rubin et al. 1984). In mammals, ankylosis is considered to be pathological, however, it is prevalent among modern fish, amphibians and reptiles (Fink 1981, Gaengler 2000) and occurs when the dentine of the tooth is fused to a surrounding layer of bone, which in turn fuses to the bone of the jaw (Peyer 1968, Luan et al. 2006, Buchtová et al. 2013). Interestingly, this condition is also prevalent throughout the fossil record of amniotes, a very large group that includes mammals (Osborn 1984, Gaengler 2000). Amniota is a formal classification that includes living and fossil members of Reptilia and Synapsida (Fig. 1), two diverse groups that diverged from a common ancestor approximately 315 million years ago (Reisz 1997). Reptilia include not only all modern birds and reptiles, but also numerous extinct groups, such as flying reptiles, aquatic reptiles and all dinosaurs. Synapsida include modern and extinct mammals as well as numerous extinct mammal relatives that are classified outside of Mammalia, collectively called non-mammalian synapsids (Fig. 1). For the rest of this study, we refer to members of this group as stem mammals, because they share a relatively close evolutionary relationship with mammals, but were not true mammals. These stem mammals resembled in many ways their distant reptilian relatives (Sidor & Hopson 1998, Kemp 2006). Palaeontologists have long recognized that ankylosis was prevalent in early reptiles and stem mammals (Edmund 1960, Peyer 1968), but their histological and developmental significance remain poorly understood, particularly in reference to the origin of the mammalian periodontium.

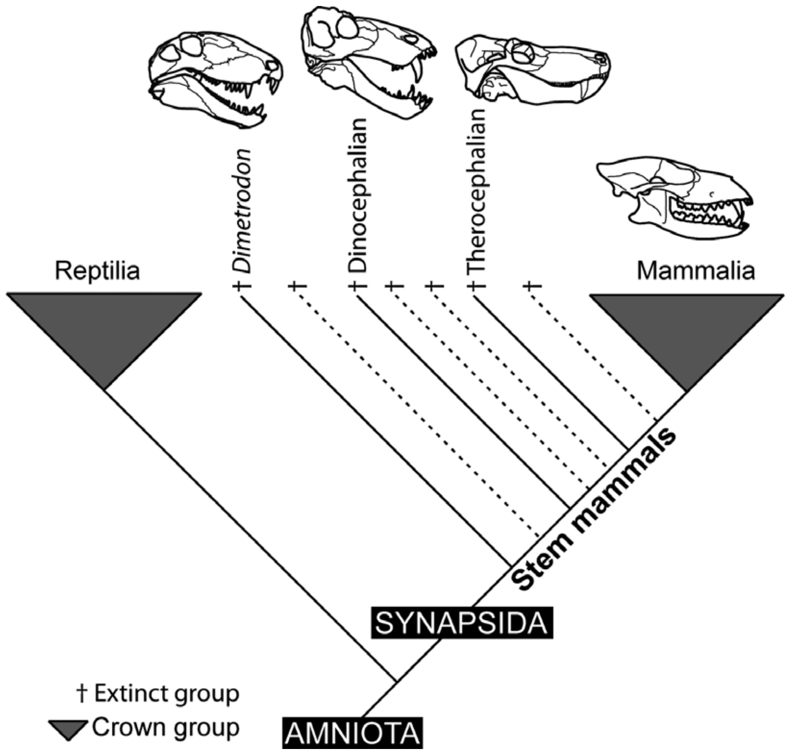

Fig. 1. Evolutionary relationships of the stem mammals (non-mammalian synapsids) and mammals within Amniota. Stem mammals are a series of extinct groups more closely related to crown (extinct and modern) mammals than to crown reptiles. Skull drawings of the three stem mammals and the crown mammal sampled for this study were modified from Brink & Reisz (2014), Kemp (2006), Sidor & Hopson (1998) and Szalay (1969). Dashed lines indicate a series of stem mammal groups that were not sampled for this study. See Sidor & Hopson (1998) for more complete evolutionary tree of Synapsida.

Here we provide the first histological examination of ankylosis in extinct animals that shared close evolutionary relationships with modern mammals (Fig. 1) and compare this condition to mammalian periodontal tissue development. Although these comparisons are made using fossils, the fact that these stem mammals constantly replaced their teeth (Edmund 1960, Osborn 1984) means that histological sections through a single jaw often preserve teeth at different developmental stages, allowing for a comprehensive look at tissue formation in fossil teeth of a single individual. We use these comparisons to show the tissue-level processes that produced dental ankylosis in these ancient relatives of modern mammals to shed new light on periodontal tissue formation and maturation in animals that possessed, but did not retain a functional periodontal ligament. For palaeontologists, interpreting the developmental significance of this type of tissue arrangement requires a better understanding of dental development, which can only be gained from reviewing empirical studies in periodontology and dentistry. Conversely, we wish to show here that integrating palaeontology and modern periodontology can provide a broader understanding of the formation and maintenance of the supporting tissues of a tooth. Palaeontological evidence is indeed a key to identifying the ancestral arrangements and developmental patterns of the periodontal tissues. Our contribution can provide direction for novel search efforts into the causes of tooth ankylosis in mammals.

Materials and Methods

Histological sections were made of the fossilized jaws of a mammal, *Hyopsodus* (specimen loaned from the National Museum of Natural History, U.S., USNM 595273), and three stem mammals: the sphenacodontid *Dimetrodon* (specimen housed at the Royal Ontario Museum, Canada, ROM 6039), an indeterminate basal therocephalian and an indeterminate basal dinocephalian (BP/1/7257 and BP/1/4851, respectively, specimens loaned from the Evolutionary Studies Institute, South Africa) for comparisons across a range of synapsids (Fig. 1). *Dimetrodon* was restricted to the early Permian period (approximately 295–270 million years ago), whereas the therocephalian and dinocephalian were from the middle Permian (Sidor & Hopson 1998). By comparison, the oldest known mammals are from the Triassic period, about 220 million years ago (Zhou et al. 2013). Thin sections were prepared in the palaeohistology laboratory at the Royal Ontario Museum following a standardized technique developed for sectioning fossil bone and teeth (LeBlanc & Reisz 2013, 2015). Each specimen was embedded in Castolite AC polyester resin and placed under vacuum for several minutes. Once the resin had set, the specimens were cut on an Isomet 1000 low-speed, wafer blade saw and mounted to frosted plexiglass slides using cyanoacrylate glue. The resin blocks were then cut off of the slide using the Isomet saw, leaving a 700-μm-thick portion of the specimen still mounted to the slide. The mounted specimen was then ground down using a Hillquist grinding machine and then hand-ground using progressively finer grits of silicon carbide powder and then polished using 1 μm-grit aluminium oxide powder and a cloth. The resulting slides were then examined and imaged using a Nikon DS-Fi2 camera mounted to a Nikon AZ-100 (Nikon Instruments Inc., Melville, NY, USA) microscope and the NIS Elements (Basic Research package) imaging software, registered to R. R. Reisz. Images were taken under plain- and cross-polarized light.

Results

Mammalian teeth

To make appropriate histological comparisons between modern mammals and their extinct synapsid relatives, we first examined the periodontal tissues of a fossil mammal, *Hyopsodus*, to determine the level of detail preserved in fossilized material (Fig. 2). *Hyopsodus* was an extinct, odd-toed ungulate mammal living in western North America during the early to late Eocene epoch, approximately 55–34 million years

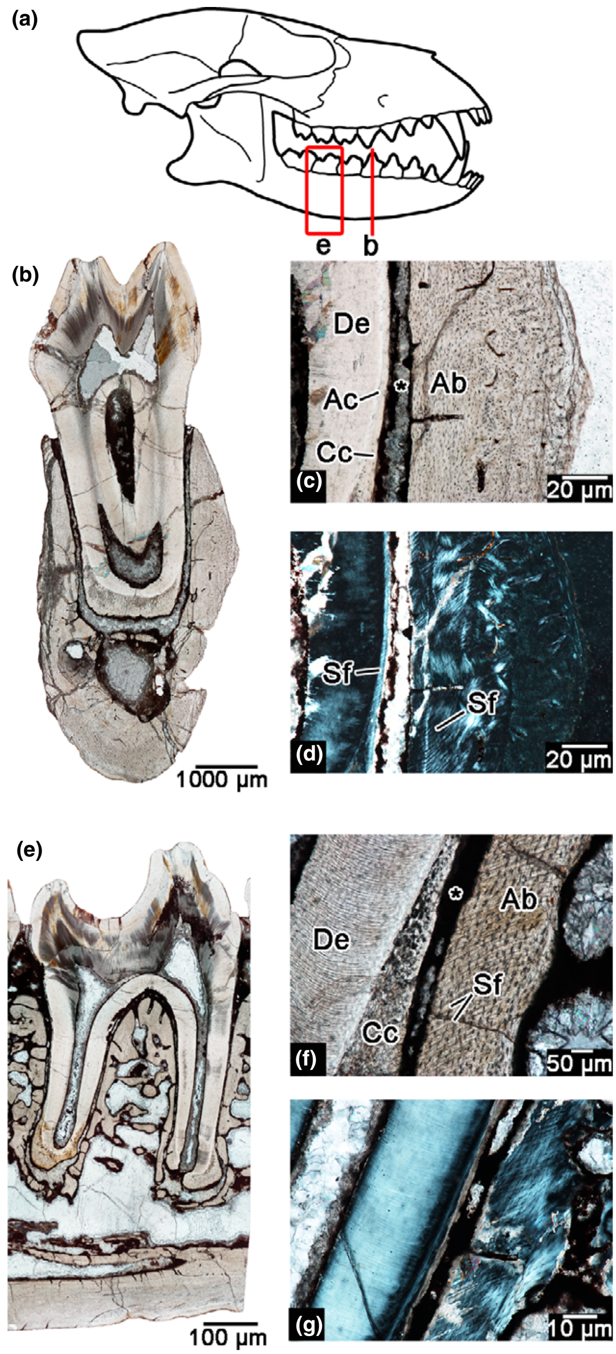

Fig. 2. Periodontal tissues in the extinct mammal *Hyopsodus*. (a) outline drawing of the skull of a fossil condylarth (modified from Szalay 1969), the extinct mammal group to which *Hyopsodus* belongs. Red lines indicate positions of sections, (b) overview image of dentary tooth sectioned in coronal aspect, (c) magnified view of periodontal tissues, (d) same view under cross-polarized light, (e) overview image of dentary tooth sectioned in parasagittal aspect, (f) magnified view of periodontal tissues in parasagittal aspect, (g) periodontal tissues under cross-polarized light. Abbreviations: Ab, alveolar bone; Ac, acellular cementum; Cc, cellular cementum; De, dentine; Sf, Sharpey's fibres. Asterisks indicate positions of periodontal space, formerly occupied by periodontal ligament.

ago (Gingerich 1974, Orliac et al. 2012, Walker et al. 2013). Thin sections of the teeth and jawbone of *Hyopsodus* reveal the presence of all periodontal tissues that are found in sections of modern mammal teeth. The dentine of the tooth roots preserves the fine dentinal tubules found in typical mammalian orthodentine. The outer surface of the dentine is coated in a thin layer of acellular tissue that we identify as acellular cementum. Acellular cementum extends from the cemento-enamel junction towards the root apex. The acellular cementum is overlain by thicker layers of cellular cementum apically (Fig. 2b–g). The cellular cementum becomes significantly thicker towards the root apex and possesses abundant cementocyte spaces, or lacunae. Although the periodontal ligament is not preserved in fossilized teeth, the resulting space left behind by the ligament, between the cementum and the alveolar bone, persists and is infilled by mineral inclusions (Fig. 2c,f). Under cross-polarized light the cellular cementum is perforated by parallel Sharpey's fibres, which represent the mineralized portions of the periodontal ligament (Luan et al. 2009, LeBlanc & Reisz 2013, Xiong et al. 2013). The surrounding alveolar bone consists of poorly vascularized lamellar bone where it meets the periodontal space. Under cross-polarized light the internal layers of this bone are perforated by Sharpey's fibres, which mark the insertion points of the periodontal ligament into the bundle bone layer lining the walls of the alveolus (Fig. 2d,g). The bundle bone layer or cribriform plate is equivalent to the alveolar bone derived from the dental follicle, which, together with the bone derived from the jaw, forms the alveolar process (Ten Cate & Mills 1972, Ten Cate 1997, Nanci 2007). Hereafter, we use the term "alveolar bone" to refer only to the bone derived from the dental follicle.

Stem mammal teeth

Superficially, most of the teeth of the stem mammals we examined appear to be ankylosed to a spongy bone tissue, which is in turn attached to the bone of the jaw (Fig. 3). Closer inspection reveals that the dentine of each tooth is coated in a thin layer of acellular tissue that is similar to acellular cementum in fossil mammals (Fig. 2c) and other reptiles (Caldwell et al. 2003, Budney et al. 2006, Luan et al. 2009, Maxwell et al. 2011a, LeBlanc & Reisz 2013). External to this tissue in each specimen is a thicker layer of cellular cementum with concentric growth lines (Fig. 3c,g,k). The cellular cementum is of variable thickness depending on the species and sizes of the teeth (Fig. 3). The outer layer of cellular cementum directly contacts a highly vascularized layer of bone in most of the specimens we examined. The cellular cementum and the alveolar bone are virtually indistinguishable from each other, due to a lack of a periodontal space. This supports previous suggestions that these stem mammals did not possess a periodontal ligament (Peyer 1968). However, under cross-polarized light, the cellular cementum is perforated by Sharpey's fibres in similar arrangements to those found in the cellular cementum and alveolar bone of mammals (Figs 2 and 3h,l). These Sharpey's fibres appear to represent the mineralized portions of the periodontal ligament that anchored to the cellular cementum of the tooth root. The spongy bone surrounding the cellular cementum consists of a primary, woven bone matrix indicative of relatively rapid formation (Gorski 1998). This bone tissue is also perforated by Sharpey's fibres with similar orientations to those found in the cellular cementum (Fig. 3d,h,i).

Although most of the teeth in the non-mammalian synapsid specimens we examined were ankylosed to the jaws, some of the individual teeth along the jaws in the therocephalian and dinocephalian were seemingly floating in a socket (Fig. 4a–d). The roots of these teeth were coated in thin layers of acellular and thicker layers of cellular cementum, but the cellular cementum did not make direct contact with the surrounding spongy bone of the socket (Fig. 4d, e). Instead, a mineral-filled space persists between these teeth and walls of the alveoli. The surrounding bone is histologically identical to the spongy bone tissue found in the ankylosed teeth. Furthermore, Sharpey's fibres are clearly visible under cross-polarized light along the outer fringes of the cellular cementum and the inner fringes of the surrounding bone. This spongy bone is likely alveolar bone, as it is identical to the dental follicle-derived alveolar bone in mammals (Fig. 3). Furthermore, we can identify two growth directions for the periodontal tissues in these stem mammals. Some of the tooth positions show extensive centripetal deposition of alveolar bone, suggesting that ankylosis was primarily accomplished by the growth of alveolar bone towards the cellular cementum and relatively minor centrifugal growth of cellular cementum (Fig. 4).

Discussion

Mammals and their synapsid relatives have the same periodontal tissues

The results of our histological examination of fossil mammals and their synapsid relatives show that it is possible to identify periodontal tissues from thin sections of fossil teeth using plain and cross-polarized light (Fig. 2). The process of fossilization erases all traces of soft tissue, including the periodontal ligament, osteocytes and cementocytes; however, the spaces left behind in the mineralized tissues provide clues as to the original composition of the periodontium (LeBlanc & Reisz 2013). On the basis of the fine detail preserved in fossilized specimens, we were able to compare the periodontal tissues of mammals to their distant relatives, the stem mammals (Figs 3 and 4). Despite the 315 million-year gap between living mammals and the oldest synapsids (Reisz 1986), we were able to find evidence for acellular cementum, cellular cementum, alveolar bone and periodontal ligament in the samples of stem mammals (contra Osborn 1984). The histological properties of the supporting tissues of the teeth across these samples were broadly similar; however, we found evidence for dental ankylosis in all of the stem mammals we examined (Fig. 3).

Ankylosis has been well-documented in non-mammalian vertebrates (Peyer 1968, Gaengler 2000); however, previous authors concluded that ankylosis occurred when the dentine of the tooth root fused with an intermediary tissue known as

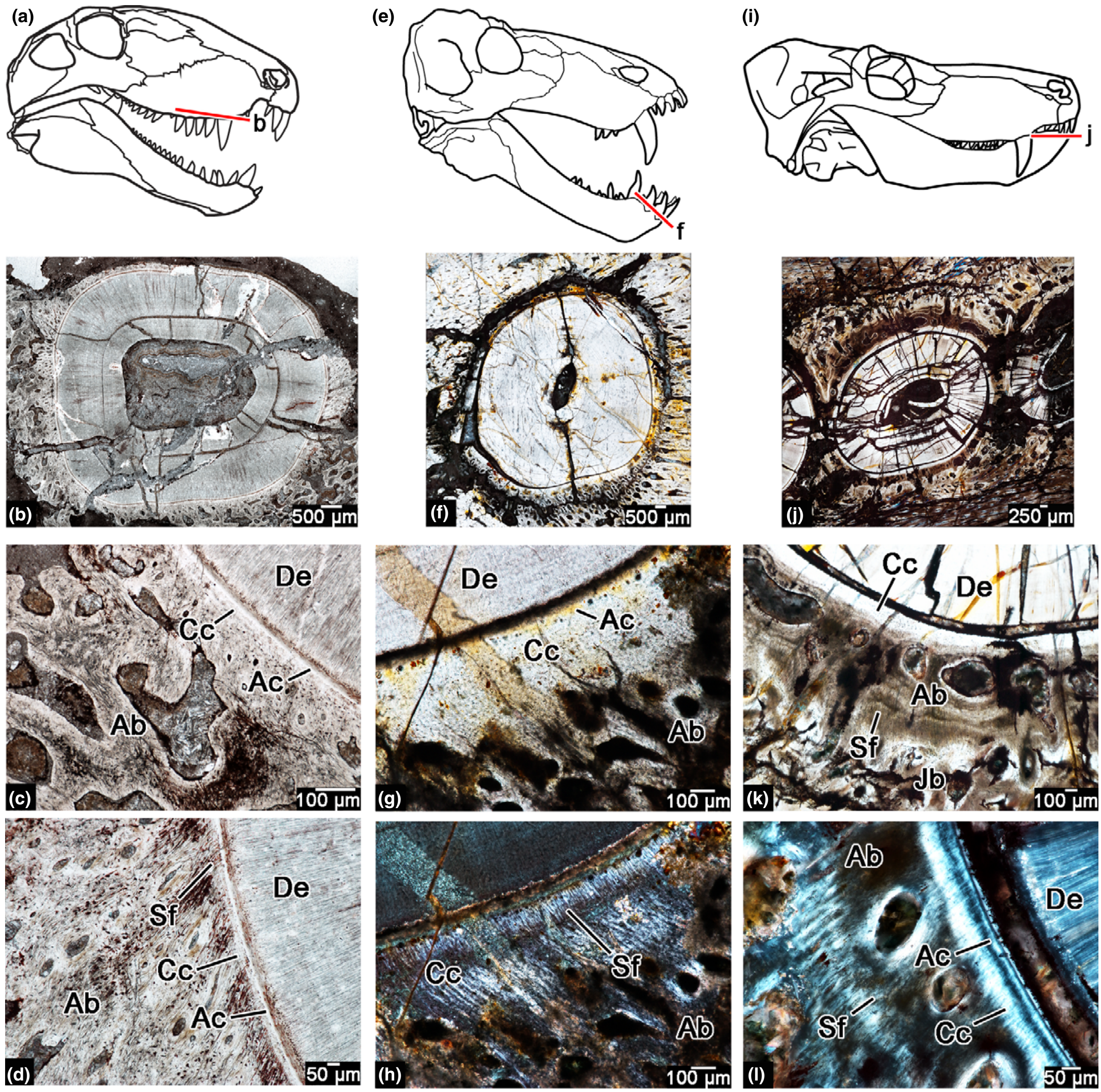

Fig. 3. Periodontal tissues in ankylosed teeth of three stem mammals. (a) skull drawing of *Dimetrodon* showing position of sections, (b) cross section of a single tooth root of *Dimetrodon* showing dental ankylosis, (c) magnified image of ankylosed tooth of *Dimetrodon*, (d) magnified image of periodontal tissues in ankylosed tooth of *Dimetrodon*, (e) drawing of the skull of a dinocephalian showing position of sections, (f) cross section of a dinocephalian tooth showing partial ankylosis, (g) magnified view of periodontal tissues in the area of ankylosis in the dinocephalian, (h) cross-polarized view of ankylosed tooth showing extensive network of Sharpey's fibres of the mineralized periodontal ligament, (i) drawing of the skull of a therocephalian showing positions of sections, (j) cross section of a therocephalian tooth showing ankylosis, (k) magnified view of periodontal tissues of an ankylosed therocephalian tooth. Note the dark colour of the alveolar bone due to the high density of Sharpey's fibres, (l) periodontal tissues of an ankylosed therocephalian tooth under cross-polarized light. Abbreviations: Ab, alveolar bone; Ac, acellular cementum; Cc, cellular cementum; De, dentine; Jb, jawbone; Sf, Sharpey's fibres.

bone of attachment (Peyer 1968, Zaher & Rieppel 1999, Luan et al. 2009, Buchtová et al. 2013). Bone of attachment has variably been interpreted as equivalent to alveolar bone, cementum or a unique attachment tissue not found in mammals (Peyer 1968, Osborn 1984, Zaher & Rieppel 1999, Luan et al. 2009). Interestingly, our thin sections of stem mammal teeth revealed dental ankylosis, but we found the same periodontal tissues as those in mammals (Figs 2 and 3). The growing consensus from more recent palaeon-

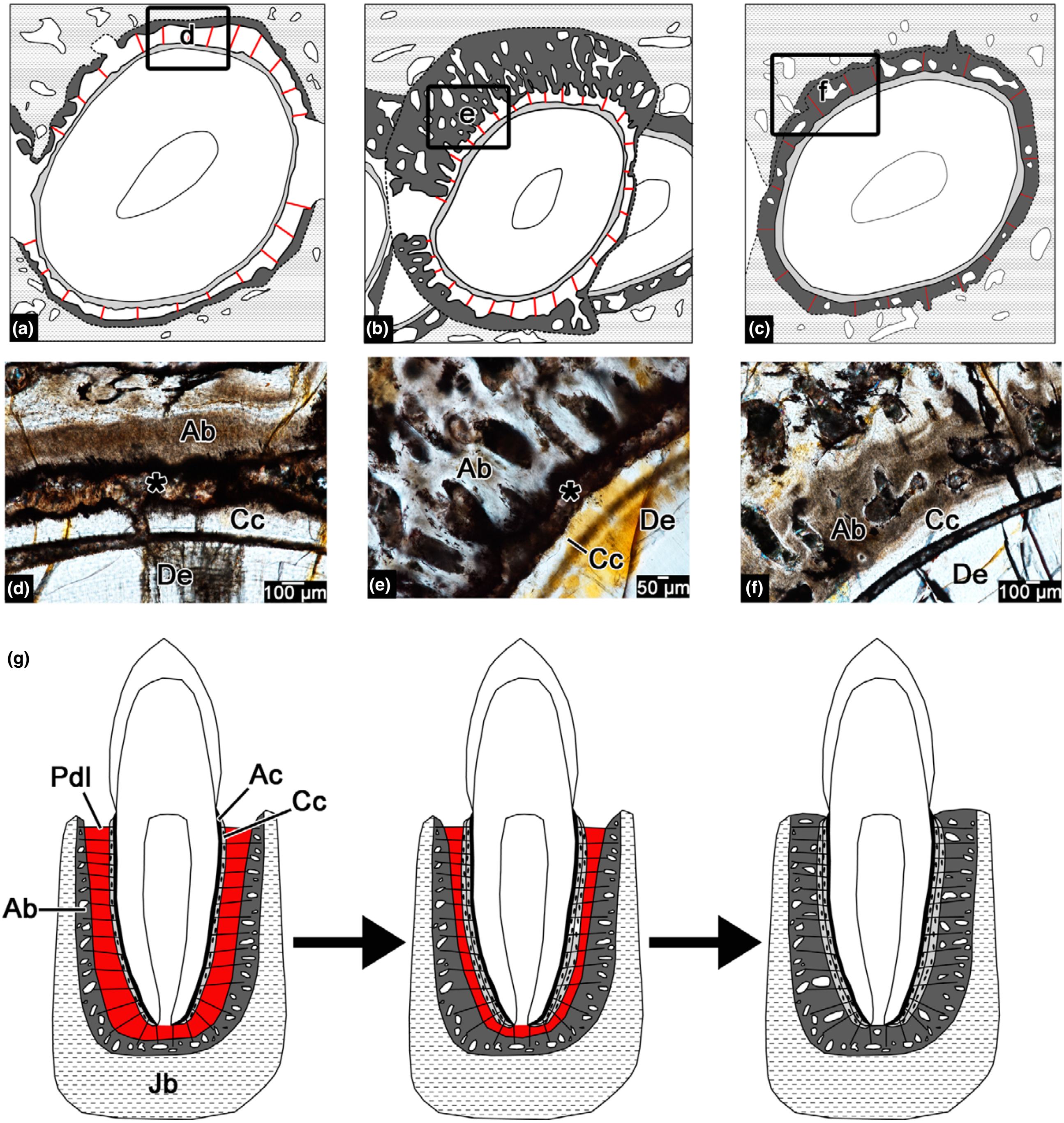

Fig. 4. Development of dental ankylosis in stem mammals based on a series of teeth along a single jaw of a therocephalian. (a) interpretation of periodontal tissues in a functional tooth that retained a gomphosis, (b) intermediate stage in which the alveolar bone (dark grey) has grown centripetally towards the cellular cementum (light grey) of the tooth root, but a periodontal ligament (red) is still retained, (c) full ankylosis of a functional tooth in which the alveolar bone contacts the cellular cementum, (d) histological section of a tooth exhibiting a gomphosis (asterisk indicates position of periodontal space). The alveolar bone's dark colour is due to a high density of Sharpey's fibres, (e) histological section of a tooth at an intermediate stage showing a reduced periodontal space (asterisk) and extensive alveolar bone deposition, (f) histological section of an ankylosed tooth with no periodontal space, (g) model for development of ankylosis in stem mammals showing eventual loss of the periodontal space by minor centrifugal deposition of cellular cementum and extensive centripetal deposition of alveolar bone. The resulting ankylosis produces alveolar bone that is perforated by extensive networks of Sharpey's fibres from a completely mineralized periodontal ligament. Abbreviations: Ab, alveolar bone; Ac, acellular cementum; Cc, cellular cementum; De, dentine; Jb, jawbone; Sf, Sharpey's fibres.

tological studies is that cementum, alveolar bone and periodontal ligament are primitively present in all amniotes (reptiles and mammals) based on the identification of these tissues and associated Sharpey's fibres in numerous extinct and living reptiles, including lizards (Caldwell et al. 2003, Budney et al. 2006, Luan et al. 2009, Maxwell et al. 2011a,b, Pretto 2012, LeBlanc & Reisz 2013, 2015). A tripartite periodontium was also described for the modern trigger-fish (Soule 1969), which may indicate that the ability to produce the tissues that characterize the mammalian periodontium are deeply conserved evolutionarily.

A developmental model for non-pathological ankylosis

We interpret dental ankylosis in stem mammals as a non-pathological condition, given that most of the teeth along the jaws in several different species exhibit this form of tooth attachment. This condition is prevalent in nearly all stem mammal groups (Osborn 1984, Sullivan et al. 2003). Moreover, using teeth at different developmental stages along the jaws of these specimens we can construct the sequence of events leading to dental ankylosis in stem mammals. Functional teeth were probably initially attached to the socket by gomphosis (Fig. 4a,g). Teeth at this stage possess well-developed cellular cementum that is perforated by Sharpey's fibres from the periodontal ligament. The surrounding alveolar bone is also perforated by Sharpey's fibres, and a periodontal space persists between the margins of the alveoli and the tooth roots (Fig. 4d). At a later stage, the vascular alveolar bone extended centripetally, which reduced the width of the periodontal space (Fig. 4b,g). Ankylosed teeth show clear distinction between the cellular cementum and the alveolar bone and show that at some point in the development of the functional tooth, ankylosis occurred by continued centripetal growth of alveolar bone (Figs 3 and 4c,g). The alveolar bone and cellular cementum surrounding ankylosed teeth are perforated by extensive networks of Sharpey's fibres from the periodontal ligament, showing that the ligament is completely calcified in ankylosed teeth (Figs 3d,h,l and 4f).

Our analysis reveals that dental ankylosis occurred naturally at some predetermined, late stage in tooth development in stem mammals. This raises intriguing hypotheses regarding periodontal tissue maintenance and ankylosis in the lineage leading to mammals. The teeth of these stem mammals retained a gomphosis for some time before becoming ankylosed to the jaw by extensive deposition of alveolar bone (Fig. 4). In mammals, ankylosis is thought to be impeded by the epithelial rests of Malassez (Luan et al. 2006, Xiong et al. 2013), which are isolated remnants of Hertwig's Epithelial Root Sheath (HERS). Although there is still some debate regarding the specific role the epithelial rests of Malassez play in maintaining the periodontium, there is some evidence to suggest that they contribute to homeostasis of the periodontal ligament, which may prevent ankylosis (Luan et al. 2006, 2009, Xiong et al. 2013, Lim et al. 2014). Lindskog et al. (1985) demonstrated that replanted teeth in which the remnants of the periodontal ligament along the root surface, and presumably the rests of Malassez, had been removed often exhibited extensive alveolar bone growth, which infilled the periodontal space and eventually caused ankylosis. This and other studies suggest that the epithelial rests of Malassez may assist in preventing osteogenesis within the periodontal ligament, preventing alveolar bone from migrating towards the cementum (Luan et al. 2006, Xiong et al. 2013). Although these conclusions are based on experimental work on modern mammals, the fate of HERS and the epithelial rests of Malassez are pertinent to the discussion of dental ankylosis we observed in these extinct stem mammals.

Developmental studies have established the roles of the dental follicle, HERS and the epithelial rests of Malassez in the development of the mammalian periodontal tissues (Ten Cate & Mills 1972, Ten Cate 1997, Luan et al. 2006, Xiong et al. 2013). In mammals and crocodilians, the onset of periodontal tissue formation is linked with the breakup of HERS, the formation of epithelial rests of Malassez, and the subsequent incursion of cells of the dental follicle, which eventually give rise to cementum, periodontal ligament, and alveolar bone (McIntosh et al. 2002, Luan et al. 2006). During the development of polyphyodont teeth in modern reptiles (excluding crocodilians), the HERS remains intact throughout root development, preventing the formation of epithelial rests of Malassez and the migration of dental follicle cells (Luan et al. 2006). The maintenance of the HERS in most reptiles means that dental ankylosis occurs apically through an intermediary bone tissue, "cementoid" or "bone of attachment". This tissue directly contacts the dentine of the tooth root and begins forming at sights apical to the HERS (Luan et al. 2006, 2009). These findings provide us with two alternative hypotheses for the development of dental ankylosis in stem mammals: either the HERS remains intact throughout the life of the tooth, or the HERS eventually disintegrates completely, leaving no functional epithelial rests of Malassez.

Unfortunately, we cannot empirically test for the existence of HERS or the epithelial rests of Malassez in extinct animals; however, we found two key pieces of evidence to reject the presence of a continuous, permanent HERS in promoting dental ankylosis in stem mammals. First, we found all of the tissues that characterize the mammalian periodontium in our sample of stem mammal teeth at every developmental stage (Fig. 3). All studies of periodontal tissue formation show that the HERS must dissociate into epithelial rests of Malassez in order for these tissues to form (McIntosh et al. 2002, Luan et al. 2006, 2009, Xiong et al. 2013). Second, the teeth of stem mammals first formed a functional gomphosis (Fig. 4), where each tooth was connected by periodontal ligament to the alveolus. Presumably, this would require at least the temporary existence of epithelial rests of Malassez to maintain a periodontal ligament (Xiong et al. 2013). We here propose that dental ankylosis in stem mammals was thus accomplished by the eventual loss of functional epithelial rests of Malassez during the life of each tooth. As transient cell populations, they may have helped to maintain a temporary ligamentous connection for each tooth, but as they ceased to inhibit osteogenesis, the surrounding alveolar bone began to encroach on the periodontal space. This bone eventually contacted the cellular cementum of the tooth root and formed a stable ankylosis that did not lead to root resorption (Fig. 4). This process would then begin anew with the formation of the successive generations of teeth. During the course of the evolutionary history of mammals, it may have become more adaptive to retain a permanent gomphosis to assist with the forces of dental occlusion and thus the epithelial rests of Malassez may have become permanent components of the periodontium.

An evolutionary approach to periodontology

Our hypothetical model for tooth ankylosis represents the end stage of the normal ontogeny (life history) of a tooth in a stem mammal and possibly other more distantly related lineages (Luan et al. 2009, LeBlanc & Reisz 2013). This may have implications for our future understanding of the maintenance of the mammalian periodontium, given that it provides an evolutionary context for tooth development in the lineage to which mammals (including humans) belong (Sidor & Hopson 1998). Gaengler (2000) hypothesized that some periodontal diseases in mammals may reflect a “phylogenetic memory”, leading to formation of periodontal tissue arrangements that are more reminiscent of ancestral forms of tooth attachment. According to Gaengler (2000), these types of perturbations to mammalian periodontal tissue formation and homeostasis may represent reversions to ancestral patterns of development. Although the studies of periodontal development across species is often viewed as adding a confounding variable to experimental assays (Lim et al. 2014), we agree with Gaengler (2000) that there is potential for new insights and avenues of research from comparative and evolutionary approaches to periodontology, as shown here. The centripetal growth of alveolar bone that we noted in several stem mammals is very similar to the reduction and eventual loss of the periodontal space in mammals following disruption of the periodontal ligament (Lindskog et al. 1985, Andreasen 2012, Xiong et al. 2013). Removing the periodontal ligament from the root surfaces of mammal teeth may thus disrupt the equilibrium that the epithelial rests of Malassez maintain within the mammalian periodontium in a way that occurred naturally in the ancestors of mammals.

Understanding the development of dental ankylosis in fossil groups may also shed new light on the mechanisms of tooth eruption in mammals and their evolutionary origins. Research into mammalian tooth eruptive mechanisms has revealed a crucial role of the dental follicle, periodontal ligament and alveolar bone remodelling in pre- and post-eruptive tooth movement (Cahill & Marks 1980, Saffar et al. 1997, Craddock & Youngson 2004). In mammals, teeth continually erupt into the oral cavity, but are opposed by their counterparts from the opposing jaw ramus (Craddock & Youngson 2004). As such, the loss of a tooth may also lead to overeruption and the eventual loss of the opposing tooth. Thus, precise dental occlusion is a key factor in mammals that helps maintain the occlusal planes of the teeth, counteracting the movements generated by tooth eruption (Craddock & Youngson 2004). Most stem mammals, however, did not exhibit precise dental occlusion and instead employed simple shearing or puncturing motions with their teeth, like modern reptiles (Davis 1961, Crompton 1995). *Dimetrodon*, basal dinocephalians and basal therocephalians were no exception (Davis 1961, Brink & Reisz 2014), although more derived stem mammals (not studied in this paper) independently evolved forms of dental occlusion by expansion and elaboration of postcanine crowns (Crompton 1995, Reisz 2006). A functional gomphosis may have been required in the early post-eruptive stages of the tooth to acquire the proper positioning within the oral cavity, however, it would have been detrimental for the tooth to continue to erupt without a counteracting force from occlusion. The loss of ankylosis and maintenance of the periodontal ligament appears to coincide with the loss of continuous tooth replacement and the origin of precise occlusion in early mammals (Crompton 1995). We hypothesize that dental ankylosis in stem mammals may have served primarily to fix teeth in position following tooth eruption to allow for more efficient grasping and puncturing of food. A more thorough examination of tooth development across the transition from stem- to crown mammals is needed, however, to test this hypothesis.

The findings we have presented here may also have implications for the understanding of the ageing of the mammalian periodontium. Several authors have noted a reduction in the periodontal space with age in mice, humans and other mammals (Van der Velden 1984, Lim et al. 2014). Although a reduction in the periodontal space has not been universally demonstrated in all studies (Van der Velden 1984), it follows a similar ontogenetic trajectory to that of stem mammals. In mammals, this process has been largely attributed to increased cementum deposition, which carries on throughout the life of the tooth (Grant & Bernick 1972, Ive et al. 1980).

However, others have concluded that alveolar bone deposition contributes to reduction in the periodontal space (Xiong et al. 2013). Interestingly, recent studies have also suggested a loss of epithelial rest cells with age, which may contribute to a decrease in the width of the periodontal space (Gonçalves et al. 2008). These results provide a tantalizing link between mammalian periodontal development and the evolution of the mammalian gomphosis from the ancestral form of ankylosis that we identify here. In the larger context of synapsid and mammalian evolution, we propose that the reduction in the periodontal space probably reflects natural ontogeny, where alveolar bone and cementum deposition normally continues until the periodontal space has been obliterated. We have demonstrated here that ancestrally, the periodontal ligament had a high osteogenic potential and was prone to calcification by means of increased alveolar bone deposition (Fig. 4). This mode of dental ankylosis was prevalent over the 300 million-year evolutionary history of synapsids and although rare in most extant mammals, this process may simply be protracted with age, such that the form of ankylosis that was common to stem mammals is delayed ontogenetically in modern mammals, only to occasionally reappear as a pathology.

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Clinical Relevance

Scientific rationale for the study: Palaeontology can provide new insights into the origin and development of the mammalian periodontium by bringing an evolutionary context to periodontology.

Principal findings: The distant ancestors of mammals exhibited a non-pathological form of ankylosis, whereby the alveolar bone eventually enclosed the periodontal space and contacted the root cementum. Calcification of the periodontal ligament was the normal condition prior to the evolution of the mammalian gomphosis.

Practical implications: age- and pathology-related changes to the mammalian periodontium mimic the ancestral pattern of ankylosis in their ancestors, suggesting that the periodontal ligament has a high osteogenic potential that is suppressed in the healthy mammalian periodontium.