Origins of bone repair in the armour of fossil fish: response to a deep wound by cells depositing dentine instead of dermal bone

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The outer armour of fossil jawless fishes (Heterostraci) is, predominantly, a bone with a superficial ornament of dentine tubercles surrounded by pores leading to flask-shaped crypts (ampullae). However, despite the extensive bone present in these early dermal skeletons, damage was repaired almost exclusively with dentine. Consolidation of bone, by dentine invading and filling the vascular spaces, was previously recognized in *Psammolepis* and other heterostracans but was associated with ageing and dermal shield wear (reparative). Here, we describe wound repair by deposition of dentine directly onto a bony scaffold of fragmented bone. An extensive wound response occurred from massive deposition of dentine (reactionary), traced from tubercle pulp cavities and surrounding ampullae. These structures may provide the cells to make reparative and reactionary dentine, as in mammalian teeth today in response to stimuli (functional wear or damage). We suggest in *Psammolepis*, repair involved mobilization of these cells in response to a local stimulatory mechanism, for example, predator damage. By comparison, almost no new bone is detected in repair of the *Psammolepis* shield. Dentine infilling bone vascular tissue spaces of both abraded dentine and wounded bone suggests that recruitment of this process has been evolutionarily conserved over 380 Myr and precedes osteogenic skeletal repair.

1. Introduction

*Psammolepis* is a large fossil jawless fish (Heterostraci), with extensive bony shields covering the head and body dorsally and ventrally (distinguishing between cranial and postcranial is not possible, see the electronic supplementary material), with scales covering the rest of the body and tail. This dermal bone, made of aspidin, lacks the osteocyte lacunae of cellular bone [1–3]. Spongy bone with large vascular spaces becomes compact as aspidones form around small central vascular canals. Both are thickened by deposition of successive laminae, formed as appositional bone grows onto all surfaces (figure 1b,j). The dense ornament of tubercles is a regular dentine tissue, deposited by odontoblasts leaving characteristic tubule trails into the pulp cavity (figure 1c). However, in older specimens, dentine also invades spaces in the vascular bone below, secreted as odontoblasts migrate away from the superficial dentinal pulp, as indicated by tracks recording their origins and paths through the bone [1–5]. This migration was previously recognized in *Psammolepis* [4] and other heterostracans as a protective (prophylactic)...
Figure 1. Psammolepis alata?, Middle Devonian Gauja Formation, Estonia. GIT100-4, except (a) Psammolepis venykovi, GIT100-7 bone surface with packed dentine tubercles and surrounding pores; Psammolepis paradoxa, GIT100-6 (c) section showing undamaged layer of regular dentine tubercles, open pores and ampullae between (arrow, white outlines); (d–i) dentine tissue from abraded tubercles compared with that in adjacent wound repair (k–r). Backscattered electron images (a,h–j), photomicrographs (c,d,e,k–n), Nomarsky (l), polarized light with gypsum plate (m), reflective confocal microscopy (f,g,o,p), surface illumination (q,r). (b,j) Overlay colours compare tissue structure in normal and abraded surfaces, primary tubercles are overlain by secondary tubercles (sup.tub.); (j) worn surface, down to primary tubercles and ampullar infill. Primary tubercular dentine pink, tertiary invasive dentine rose, dentine filling ampullae mauve, vascular spaces purple. Tubercular dentine above compact bone (com. bo), dentine infilled, regular ampullar spaces below tubercles (1° dent). (d,e) Worn tubercles and tertiary dentine indicate fields (f) dentine tubules of tubercle arising from narrow vascular canal (vc), (g) similar tubule ‘sprays’ from tertiary dentine. (h) Characteristic primary dentine (1° dent) with incremental lines parallel to dentine surface (arrows), tubules normal to these, cell process spaces with post-mortem crystal infill show these tubules run through secondary (2° dent) and tertiary (3° dent) dentine, surrounding compact bone. (i) Sharpey’s crystal fibre bundles (cfb) normal to laminae with correspondingly less mineral at the arrest lines (arrows). (k) Vertical section through wound repair, compact mass sealed by translucent layers (arrows), spongy bone below. (l,m) Fields (asterix) from (n) of repair dentine, tubules sprouting from vascular canal (arrows, l), within broken compact bone as shown by alternate colours of birefringent bone laminae (arrow, m). (n) Lower wound tissue (above spongy bone, arrest line) shown as brown infill. Boxed areas (o,p), dentine tubules, extending from brown repair arrest line (bright) within translucent dentine layer (k). (q,r) Surface as in figure 1k, opaque wound tissue (pale yellow) and sealing dentine layer (arrows (k)), (r) vascular canals from spongy bone with arrays of dentine tubules (o,p). asp.d, aspidones; wo.rep.tiss, wound repair tissue.
mechanism to strengthen bone, responding to ongoing wear (figure 1d–h) or to skin damage and bacterial infection [3–5]. However, as described below, dentine is observed to repair substantial bone damage, for example one resulting from predator attack (figures 1k and 2a,b; [6]). In this instance, the repair is by invasive dentine produced by odontoblasts migrating deep into the disrupted, broken bone, sealing the inner and outer wound margins by a layer of dentine. Notably, there is almost no depositional response of bone cells, despite the bone comprising most of the dermal shield. Repair results from new dentine deposition, formed as odontoblasts, within the vascularized pulp migrated through tissue spaces, covering the remaining bony scaffold with dentine. Today, dentine is rare in the dermal skeleton but is present in all teeth where reaction to wear and damage in mammals involves participation of pulpal stem-niche mesenchyme including perivascular cells [7]. Based on results of the growth history of reparative dentine in the dermal armour of *Psammolepis*, we propose that in life mobilization of similar cells from tubercle pulps and peritubercular, flask-shaped crypts (ampullae; figure 1a–c) initiated dentine secretion in response to moderate minor (reparative) and gross bone damage (reactive). We suggest that the process of wound repair by dentine (in teeth) first evolved over 380 Ma in the dermal skeleton of jawless fishes, for instance *Psammolepis*. Only in more derived vertebrates, repair by bone cells replaced dentine repair [8].

Figure 2. *Psammolepis alata?*, GIT 100-4, Middle Devonian Gauja Formation, Estonia. (a) Macrophotograph, dermal shield wound in dorsal view. (b–i) Backscattered electron micrographs, (b) complete section; (c) transmitted light micrograph, dentine tubules black in repair tissue surrounding sand grains; (d) same vascular canal (c) in reflective scanning mode, short tubules branch from vascular canal, lower wound area showing broken bone (arrows) and invasive dentine (38 dent); (e) area of broken bone and dentine deposition along bone scaffold (arrows); (f) dentine deposited on broken bone surfaces (arrows), indicated by presence of tubules; (g) lower area of wound repair showing dentine deposited (arrows) on broken bone, arrow head indicates open space and location of odontoblasts; (h) lower area of wound repair showing dentine deposited around broken bone pieces (arrows); (i) sand grains enclosed in repair dentine; amp, ampullae; bo, bone (aspidin); com.bo, compact bone; spo. bo, spongy bone; sup. tub, superficial tubercles; wo.rep.tiss, wound repair tissue.
2. Material and methods

Psammolepis specimens were collected from the Gauja Formation (Givetian, Middle Devonian), Piusa River, Kalmumägi (Jõksi) locality, Estonia and Braslau River locality, Latvia. Wound repair tissue in the dermal shield was examined from sections and adjacent polished surfaces for backscattered electron imaging. Energy dispersive X-ray spectroscoiy produced simultaneous micrographs and elemental X-ray emission spectra (see electronic supplementary material, figure S1b-c) to estimate mineral composition (post-mortem and biological). Photomicrographs were taken using Nomarski optics; polarized light with gypsum plate shows alternate positive and negative birefringence of mineralized collagen fibres running parallel in each growth layer of bone; reflective confocal scanning recorded as the brightest pixel trace from a z-stack of images shows infilling in dentine tubules.

3. Results: observations and interpretation

The wound in the Psammolepis bony shield forms a rounded pit at the surface, infilled by repair tissue and surrounded by intact but worn tubercular ornament (figures 1k and 2n,b). Our new observations compare invasive dentine growth into vascular spaces around and below the tubercles (e.g. described in [3–5]) with the previously unidentified wound repair tissue. This allows tissue type, dentine and bone (aspidin) and their independent growth histories to be distinguished, irrespective of topography (figures 1 and 2).

(a) Dentine tissue

Normally, cell processes maintain living dentine within associated tubules throughout life. Pulpal cells respond to changes at the surface by further dentine deposition, forming secondary and tertiary dentine. Primary tubercles are made of regular dentine with tubules (odontoblast cell process spaces) extending from an open pulp in Psammolepis (figure 1c). These tubules identify secondary and tertiary dentine deposition by their continuous course from their origin to end of cell migratory routes (figure 1d–h). This and the growth lines (sequential incremental lines) show how the direction of odontoblast migration through soft tissue spaces produced invasive dentine, including that onto damaged bone tissue. Odontoblast tubules cluster in closed-packed arrays from vascular canals below the dentine tubercle (figure 1d–h). Tissue dentine is identified independent of the topographical location, by tubules infilled with post-mortem crystals in contrast with their absence in bone tissue (see electronic supplementary material, figure S1b,c).

(b) Bone tissue

Neither cell spaces nor canaliculi (osteocyte bone) are found in aspidin, but densely mineralized crystal bundles aligned to original fibre direction (Sharpey’s fibres) are incorporated into the bone matrix, perpendicular to the bone laminae (see electronic supplementary material, figure S1c,p). These collagen bundles (secreted by fibroblasts) identify bone tissue growth as they continue across successive laminae with mineral growth phases that coincide with the arrest lines of collagen laminae, secreted by formative bone cells (osteoblasts).

(c) Dermal tissue

Dentine tubercles on new, unworn surfaces include pores between them that lead into ampullae of the vascular tissues. Dentine tubercles on new, unworn surfaces are formed close together and include pores between them that lead into ampullae of the vascular tissues (figure 1a–c). These primary tubercles are superimposed by secondary tubercles (figure 1b,d,e). In all layers, ampullae alternate with tubercles, with pulps and soft tissue ampullae infilled by tertiary dentine (arrows, figure 1b). The compact structure of bone below the tuberculated surface (either side of the wound) is achieved by not only deposition of successive bone layers to earlier spongy bone surfaces (aspidones) but also dentine infilling vascular tissue spaces (figure 1b,j, superimposed colours). Both show the direction of growth with sequential arrest lines in the bone and pauses in dentine secretion (figure 1b,i). Below the compact bone is spongy bone with large vascular spaces, within which formative surfaces of tertiary dentine are seen (figure 1b,j, purple zones, asterix).

(d) Wound tissue

The central part of the wound comprises a dense, mixed tissue of broken bone enmeshed within dentine, incorporating quartz grains buried by the new tissue (figures 1i–q and 2). The wound repair tissue is surrounded above and below by continuous, translucent dentine layers. In the wound mass, dentine tubules emerge from vascular canals (figures 1l,j,p and 2c,d), and fractured bone surfaces are generally coated with new dentine (figure 2e–g) comparable with the intimate bonding of invasive dentine to the bone below surface wear (figure 1d–h,j). The random mixture of dentine and bone is distinguished by dentine tube bunches originating from vascular spaces (figure 1e,r), tubules extending for long distances; the bone by its laminated structure and perpendicular fibre bundles (figure 1i,m; electronic supplementary material, figure S1c).

The dense glassy layer below this chaotic wound tissue is dentine with tubules branching in clusters (figure 1k,u–r), emerging from vascular canals linking the reactionary dentine with spongy bone. This sealing layer is dentine still growing into the spongy bone below before death, with active forming surfaces in the soft tissue spaces (figure 2f,g; arrow head, wound tissue mineralogy described in the electronic supplementary material).

4. Discussion: developmental model

We propose a developmental model in which, by comparison with reactionary dentine in mammalian teeth, mesenchymal cells are mobilized to form odontoblasts, depositing dentine to repair wounds in the early vertebrate bony skeleton (figure 2h–g). In all teeth, dentine-producing odontoblasts are known to be neural crest (NC; ectomesenchyme) derived [9–11]. Ectomesenchyme was thought to contribute to the development of the early vertebrate dermal skeleton, because the dentine tissue is identical to that of teeth and believed to be homologous in all vertebrates [12]. Recent research has questioned the contribution of NC to dermal bone (e.g. [9] versus [13]), but importantly, the NC contribution to the skeletal dentine has not yet been tested (see the electronic supplementary material). Until then, we accept that NC contributes to dentine in the early vertebrate dermal skeleton.

Skeletal tissues in taxa, for instance Psammolepis, preserve characters associated with their living state, recording ghosts of cells as spaces and cell activity as incremental growth lines. Observations on these tissues allow us to propose our
developmental model that, by comparison with homologous extant tissues, defines the location of regeneration of dentine-producing cells. This palaeobiological model suggests activation of odontoblasts from ampullae and tubercles (figure 1b). Dentine extended its distribution from the tubercle pulp to ampullar spaces alongside the tubercles (coloured overlays, figure 1b), as shown by younger, non-superimposed and unworn tubercles, where pulp cavities and ampullae are not filled with dentine (figure 1c). In older tissue, dentine spread throughout the bone from these sites, leaving trails of tubules with incremental lines showing directional growth. Regenerated dentine arose in response to surface wear and notably to repair a wound. This facilitated inflow of the vascular spaces to strengthen dermal bone and then repair a wound, both through extensive dentine secretion (figure 2c–h).

In teeth of living mammals, odontogenic cells that can be mobilized to form additional dentine in response to wear and damage are housed in the tooth pulp and surrounding vascular tissues. Pulpal mesenchymal stem cells (MSC) and pericytes associated with blood vessels are included in the MSC niche and can respond to appropriate stimuli [7]. In response to damage, pericyte number is greater than during continuous growth of the rodent dentition in response to functional wear, indicating the adaptation of an existing mechanism of reparative for reactionary dentine in response to more extensive damage. We suggest that a similar adaptation occurred in Psammolepis, with response to wear of dentine tubercles co-opted for rapid wound repair. Comparable vascularized regions are associated with the Psammolepis dermal tubercles, where odontoblasts continued their activity to deposit tertiary dentine, including three sites: tubercle pulp, ampullar space and spongy bone. Although ultimately untestable in fossils, we suggest that ectomesenchymal stem-niche cells with odontogenic potential were located in these areas ([4]: figures 10–13). Thus, odontoblasts were able to repair the bone within a wound, with massive amounts of reactionary dentine deposited onto all surfaces of broken bone (figure 2c–g), none showing comparable repair by bone deposition.

We suggest that peritubercular pores leading to ampullae were part of a vascular canal network arranged within the soft tissue of heterostracan dermal bone. Here, odontoblastic cells for regeneration of reparative and reactionary dentine were activated to repair damage of both minor and major kinds. This odontogenic repair mechanism in teeth of living animals first evolved in jawless fishes over 380 Ma, before osteogenic bony repair seen, for example in tetrapods (see the electronic supplementary material).

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