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Mineralized cartilage in the skeleton of chondrichthyan fishes

Mason N. Dean, Adam P. Summers*

Ecology and Evolutionary Biology, University of California – Irvine, 321 Steinhaus Hall, Irvine, CA 92697-2525, USA

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Abstract

The cartilaginous endoskeleton of chondrichthyan fishes (sharks, rays, and chimaeras) exhibits complex arrangements and morphologies of calcified tissues that vary with age, species, feeding behavior, and location in the body. Understanding of the development, evolutionary history and function of these tissue types has been hampered by the lack of a unifying terminology. In order to facilitate reciprocal illumination between disparate fields with convergent interests, we present levels of organization in which crystal orientation/size delimits three calcification types (areolar, globular, and prismatic) that interact in two distinct skeletal types, vertebral and tessellated cartilage. The tessellated skeleton is composed of small blocks (tesserae) of calcified cartilage (both prismatic and globular) overlying a core of unmineralized cartilage, while vertebral cartilage usually contains all three types of calcification.

Keywords: Elasmobranch skeleton; Mineralization; Calcified cartilage; Tesserae

Introduction

The breadth of morphological variation of the mineralized cartilage of the endoskeleton of chondrichthyan fishes (sharks, rays, and chimaeras) has led to a confusion of terminology in the literature. Conflicting descriptive terms make it difficult to define, let alone answer, questions of the evolution, homology and function of the skeleton and skeletal tissues. Here we lay out the most accepted terminology for mineralized tissue, sometimes called 'calcified cartilage' in the literature, in cartilaginous fishes and propose a hierarchical framework for future descriptive work.

To integrate the convergent evolutionary (Smith and Hall, 1990; Sansom et al., 1992; Hall, 2005), paleontological (Coates et al., 1998; Janvier et al., 2004), developmental (Davis et al., 2004) and biomechanical (Summers, 2000; Schaefer and Summers, 2005; Dean et al., 2006) interests in cartilaginous skeletons, we need a common language. Furthermore, the current terminology masks unappreciated complexity in morphology that will fuel future research in several of these fields.

Classification of elasmobranch cartilage tissue types

The entire endoskeleton of sharks, chimaeras and rays is cartilaginous, composed of chondrocytes in an extracellular matrix (ECM) surrounded by a fibrous perichondrium. The ECM may be mineralized to varying degrees with crystals of calcium phosphate hydroxyapatite (Applegate, 1967; Dean et al., 2005). There are no nanostructural data on the orientation or size of these crystals, but the higher-level organization of the mineral has inspired several useful descriptive terms.

*Corresponding author.
E-mail address: asummers@uci.edu (A.P. Summers).

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From these terms and concepts we recognize three types of calcification (synthesized originally by Ørvig, 1951) in a natural classification based on anatomy and location, that will provide a framework for appreciating the true complexity at this level of organization.

Microstructure: calcification types

Areolar calcification (*var.* alveolar, Moss, 1977) is a densely calcified tissue that occurs in the vertebral centra of most cartilaginous fishes, forming what has been called the 'double cone' of the vertebral body (Ridewood, 1921; Ørvig, 1951) (as illustrated by the hemisected centra in Fig. 1a). This form of mineralization is laid down in concentric rings and has been successfully used to age cartilaginous fishes (Jones and Geen, 1977; Lessa et al., 1999).

Prismatic calcification is always perichondrally associated (Fig. 1b) and is so dense as to specularly refract light (Wurmbach, 1932; Ørvig, 1951).

Globular calcification (*var.* spherulitic, e.g., Ørvig, 1951; Janvier et al., 2004) is a moderately well mineralized tissue formed of nanoscale (40–55 nm) spherules of hydroxyapatite fused together (Fig. 1c) (Ørvig, 1951; Dean et al., 2005).

Mesostructure: tesserae

Prismatic and globular calcification (Figs. 1b,c) typically co-occur in blocks of calcified tissue called *tesserae* that form a continuous mosaic between the perichondrium and uncalcified core of most endoskeletal elements (Fig. 2). The gradation within a single tessera from globular calcification on the inner surface to prismatic calcification on the perichondral surface (Figs. 1b,c and Fig. 2 lower inset) raises the possibility that they represent ontogenetic stages of progressively more mineralized tissue, though there is no published developmental evidence (Ørvig, 1951; Moss, 1977; Kemp, 1979). It has also been proposed that these two forms of calcification are of completely different cellular

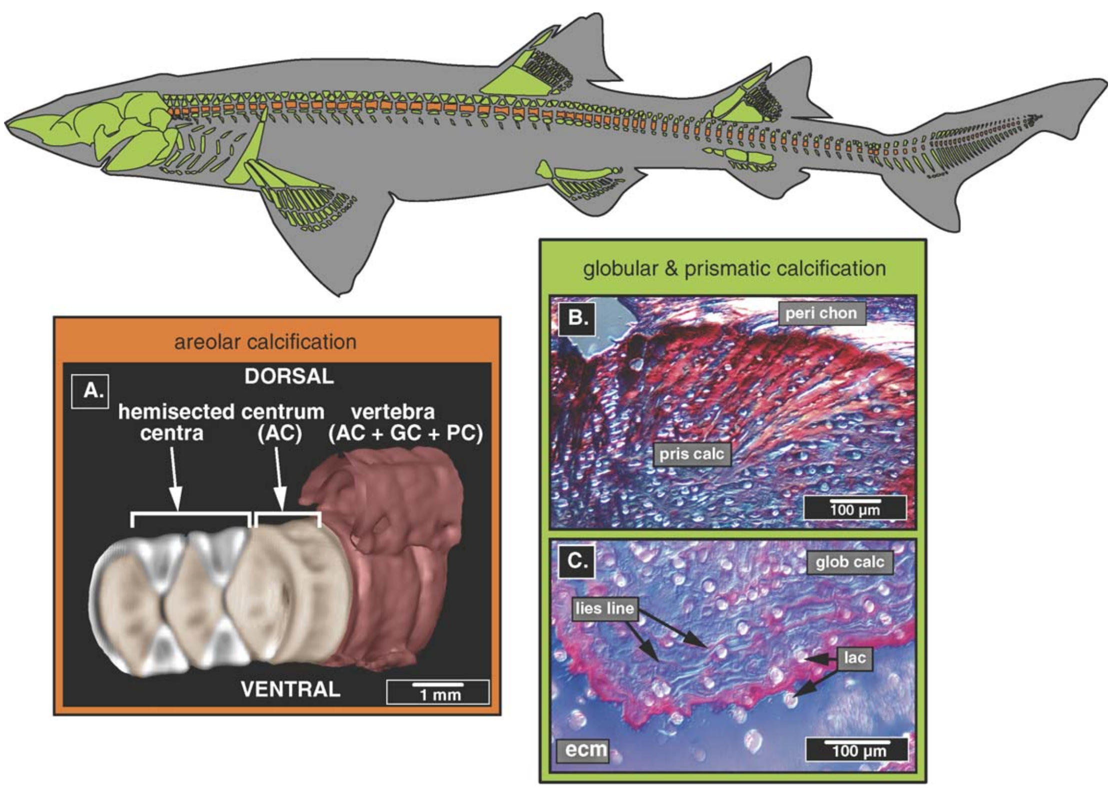

Fig. 1. Schematic of a generalized shark endoskeleton showing occurrences of areolar (a), prismatic (b) and globular (c) calcification types. Prismatic (pris calc, PC) and globular calcification (glob calc, GC) form tessellated skeletal tissue in all areas color coded green, while areolar calcification (AC) forms the centra of the vertebral column, color coded orange. The majority of the skeleton is *tessellated cartilage*, comprised of a cortex of mineralized blocks (tesserae), lying just beneath the fibrous perichondrium (peri chon) and overlying the uncalcified ECM. Prismatic and globular calcification, respectively, form the outer and inner surfaces of 'surface' tesserae (see text). Calcification is apparently periodic, entombing chondrocytes in their lacunae (lac) and forming concentric Liesengang lines (lies line). Prismatic and globular calcification often ornament the vertebral structures surrounding areolar-calcified vertebral centra; this interaction of all three calcification types is apparently only found in the *vertebral cartilage* of cartilaginous fishes (a).

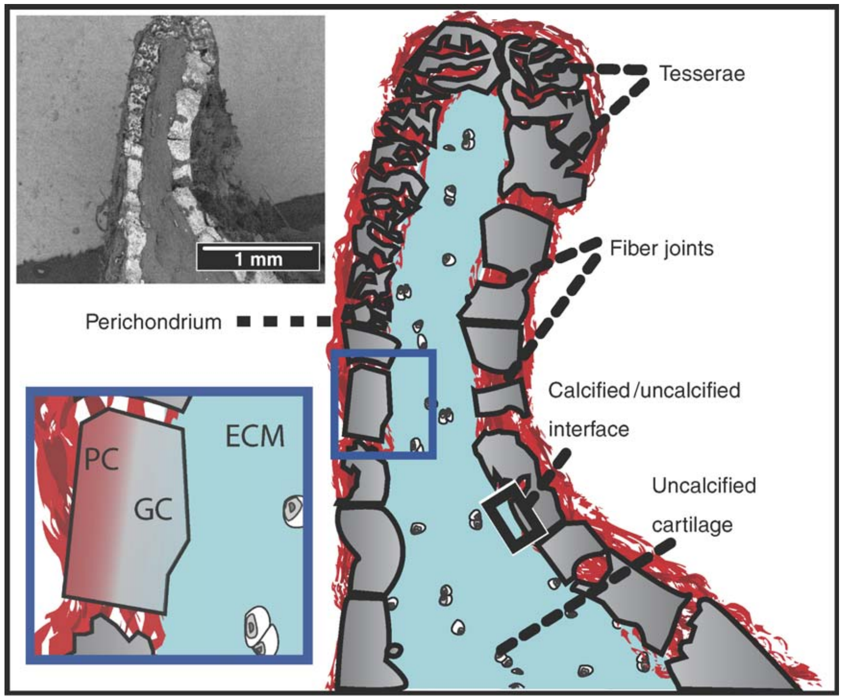

Fig. 2. Uncalcified and calcified phases of tessellated elasmobranch cartilage, depicted as a schematic of a cross-sectional backscatter electron micrograph of the lower jaw of the round stingray, *Urobatis halleri* (upper inset; white regions are calcified tissue). The lower inset provides an expanded view of the region bounded by the blue box, showing the arrangement of prismatic (PC) and globular (GC) calcification relative to the uncalcified cartilage matrix (ECM) and fibrous perichondrium.

origin, with prismatic and globular calcification being chondrally and perichondrally derived, respectively (Kemp, 1977; Kemp and Westrin, 1979). Chondrocytes on the inner side (i.e. adjacent to unmineralized ECM) of tesserae are still alive and surrounded by characteristic 'Liesengang lines' (Fig. 1c) that signify varying density of calcification (Applegate, 1967). Some authors (e.g., Moss, 1977) believe entombed chondrocytes to be vital in both prismatic and globular types, while others (e.g., Summers, 2000) only observe them in the latter.

Macrostructure: skeletal types

The three calcification types are associated with uncalcified cartilage and organized into two skeletal types: vertebral cartilage and tessellated cartilage (Fig. 3).

Vertebral cartilage can contain all three calcification types (illustrated by the vertebra in Fig. 1a). Areolar cartilage comprises the centrum ('double cone') of each vertebra, with all surrounding structure (e.g., neural/hemal arches, the vertebral body) formed by uncalcified cartilage, typically sheathed with tesserae. In addition to forming the chondral surface of tesserae, globular calcification may also be present on the outer surface of the centra (Fig. 1a) (Ridewood, 1921; Clement, 1992). While the areolar part of the centrum is well known in sagittal section by biologists interested in aging sharks, there is nothing known of the mechanics of the centra, nor of the interaction between the different forms of calcification found in the rest of the vertebral cartilage (Summers and Long, 2006). There is extensive morphological variation in calcification at the level of species, but there has been no examination of this variation in an evolutionary context (Hasse, 1879; Ridewood, 1921; Ørvig, 1951).

Tessellated cartilage forms the remainder of the axial and the appendicular skeleton and consists of a mosaic of tesserae (composed of both prismatic and globular calcification) overlaying a core of unmineralized ECM (Kemp and Westrin, 1979) (Fig. 2). The arrangement of tesserae appears to be functionally important in stiffening the skeleton with several layers present at the corners of the jaws in large sharks and throughout the core of jaws of species with highly demanding feeding modes (e.g., durophagy, prey excavation) (Dingerkus et al., 1991; Summers, 2000; Dean et al., 2006). Where there are multiple tesseral layers, 'surface' and 'sub-surface' tesserae exhibit different calcification types, perhaps reflective of their tissue associations. 'Sub-surface' tesserae do not have contact with the perichondrium and do not have any prismatic calcification but rather are composed entirely of globular calcification (Summers, 2000). This raises the possibility that the perichondrium is either the origin of, or induces the

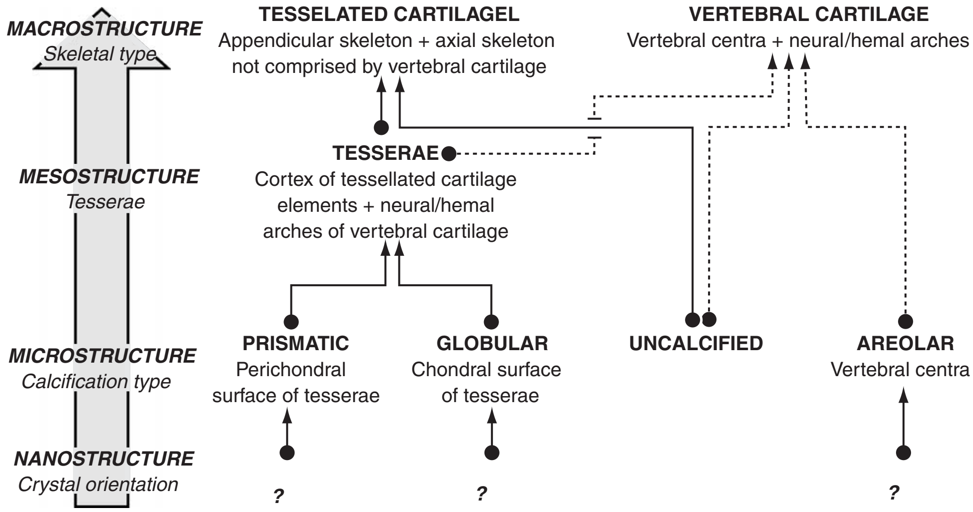

Fig. 3. Distribution of types of cartilage and mineralization in sharks and rays.

appearance of, prismatic calcification (Kemp and Westrin, 1979). From thin sections or micro-CT scans of fossil material it should be possible to distinguish sub-surface tesserae from the more common perichondrally associated ones.

Discussion

The development of tesserae and the role of the uncalcified inner core are of particular interest because, in contrast to endochondral bone, the signaling that produces this calcification happens in the absence of any vascular tissue. Though all extant sharks and rays have tessellated skeletons, the evolutionary history of this character is unknown. Since bone has been secondarily lost in sharks the history of the tessellated skeleton is of considerable functional importance because of selective pressures to maintain a stiff skeleton (Coates et al., 1998).

Previous studies have ignored the interaction of different calcification types by equating 'tesserae' with 'prismatic calcification' or layering of 'prismatic calcium phosphate' (Ørvig, 1951; Applegate, 1967; Cappetta, 1987; Dingerkus et al., 1991; Currey, 2002; Hall, 2005) or referring to the entire skeleton as 'prismatic' (Halstead, 1974). Tesserae contain two types of calcification; Kemp and Westrin (1979) made a vital contribution by recognizing the association between globular and prismatic calcification in tesserae. To speak of chondrichthyan skeleton as 'prismatically calcified' is to refer only to the perichondral portion of surface tesserae, ignoring sub-surface tesserae as well as vertebral cartilage.

To further clarify the situation we have described four distinct levels of organization in the calcified portions of the chondrichthyan skeleton–hydroxyapatite crystal orientation and size (nanostructure); calcification type (microstructural arrangement and density of mineral phase); tesserae (mesostructure); and skeletal type (macrostructural arrangement and association of calcification types) (Fig. 3). While the first of these levels has not been investigated at all, the third and fourth levels of organization have the most immediate potential to inform our understanding of the evolution of the vertebrate endoskeleton.

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