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REVIEW

The enigmas of bone without osteocytes

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One of the hallmarks of tetrapod bone is the presence of numerous cells (osteocytes) within the matrix. Osteocytes are vital components of tetrapod bone, orchestrating the processes of bone building, reshaping and repairing (modeling and remodeling), and probably also participating in calcium-phosphorus homeostasis via both the local process of osteocytic osteolysis, and systemic effect on the kidneys. Given these critical roles of osteocytes, it is thought-provoking that the entire skeleton of many fishes consists of bone material that does not contain osteocytes. This raises the intriguing question of how the skeleton of these animals accomplishes the various essential functions attributed to osteocytes in other vertebrates, and raises the possibility that in acellular bone some of these functions are either accomplished by non-osteocytic routes or not necessary at all. In this review, we outline evidence for and against the fact that primary functions normally ascribed to osteocytes, such as mechanosensation, regulation of osteoblast/clast activity and mineral metabolism, also occur in fish bone devoid of these cells, and therefore must be carried out through alternative and perhaps ancient pathways. To enable meaningful comparisons with mammalian bone, we suggest thorough, phylogenetic examinations of regulatory pathways, studies of structure and mechanical properties and surveys of the presence/absence of bone cells in fishes. Insights gained into the micro-/nanolevel structure and architecture of fish bone, its mechanical properties and its physiology in health and disease will contribute to the discipline of fish skeletal biology, but may also help answer questions of basic bone biology.

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The Osteocyte

Osteocytes—one of the three primary cell types found in vertebrate bone—have a pivotal role in the biology of the skeleton, although some of their purported functions are still debated and intensively investigated (for thorough reviews of our current understanding and debates, see for example Bonewald,[1] Wysolmerski[2] and Kennedy and Schaffler[3]). Osteocytes arise from osteoblasts that have become embedded within the extracellular matrix they deposit (osteoid).[4–6] Although effectively trapped within lacunae in the mineralized matrix, osteocytes maintain a complex networking system, communicating with their neighbors and with cells on bone surfaces via long cellular processes, which extend from their lacunar cell bodies via microscopic canals (canaliculi) that perforate the bone matrix.[7–10]

The extensiveness of the lacunar-canalicular network provides support for the hypothesis that osteocytes function primarily as local sensors, triggering processes that allow bone to respond to surrounding environmental mechanical conditions or to hormonal signals during changing ion homeostasis demands. As the mechanosensors of bone, osteocytes detect local changes in strain in their vicinity,[11–13] and in response express membrane-bound proteins and release soluble factors that regulate and coordinate the function of bone surface cells (bone-forming osteoblasts and bone-resorbing osteoclasts[3,14–17]). The critical role of osteocytes in mechanosensing and bone tissue health is underlined for instance by Tatsumi *et al.*'s study,[18] where targeted ablation of osteocytes in a transgenic mouse model induced osteoporosis and a reduced response to changing mechano-environment. In addition, strong evidence is accumulating to show that osteocytes are part of the endocrine regulatory mechanism of mineral homeostasis, both locally through resorption (and perhaps reforming) of the matrix surrounding their lacuna, as well as through release of factors affecting processes in distant organs such as phosphate release or absorption in the kidney.[19–21]

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The ubiquity and abundance of osteocytes is further testament to their importance: they are extremely numerous (between 30 000 and 90 000 cells per cubic mm, and representing 90–95% of all bone cells), can be very long-lived (up to several decades in humans) and have been found in all investigated species of tetrapods (amphibians, reptiles, birds and mammals; **Figures 1a–d**).[22,23] It is therefore thought-provoking that the entire skeleton of many fishes—in particular the advanced teleost fishes, which represent at least 50% of the bony vertebrate species—consists entirely of bone material that does not contain osteocytes. This observation raises the intriguing question of how the skeleton of these animals accomplishes the various essential functions attributed to osteocytes in other vertebrates, and raises the possibility that in fish with anosteocytic bone, some of these functions are either accomplished by non-osteocytic routes or are not necessary at all. In the following sections, we address three major functions known and suggested for osteocytes—modeling, remodeling and mineral metabolism—and discuss evidence for the occurrence of these processes in anosteocytic fish bone and the implications for this evidence on the study of osteocyte and bone biology. It should be noted that although 'osteocytic/anosteocytic bone' are perhaps the more precise terms for bone with/without osteocytes, they are not used in the majority of fish skeletal biology literature; for the sake of this short review, we will employ from here on the recognized terms 'cellular/acellular bone' to allow comparison with previous works.

Acellular Fish Bone

To understand fish bone's place in the broader context of bone biology, we could first ask whether we can call this tissue 'bone' at all. Such semantic questions are in fact quite difficult to answer for vertebrate skeletal tissues, where many exceptions to 'rules' and intermediate tissue types exist. Even within tetrapods, there are several types of 'bony' tissues, ranging from the various and well-studied types of lamellar bone, to stable (nontransitional) bone–cartilage intermediates like chondroid bone, to hyperossified otic bone tissue.[4,5,24,25]

These tissues, however, are unified by their compositional features, the vast majority of which are shared by the bones of fishes: fish bones consist of the same basic building blocks as tetrapod bones (type I collagen fibers and calcium phosphate crystals).[26] Furthermore, as with mammalian bone, fish bone provides a light yet stiff material to serve as anchor points for muscles and protection for internal organs (although the fish bone tissue itself is apparently less stiff than that of mammals[27,28]). Furthermore, fish bones also contain bone-forming cells (osteoblasts), which are mesenchymaly derived, and bone-resorbing cells (osteoclasts), which are probably derived

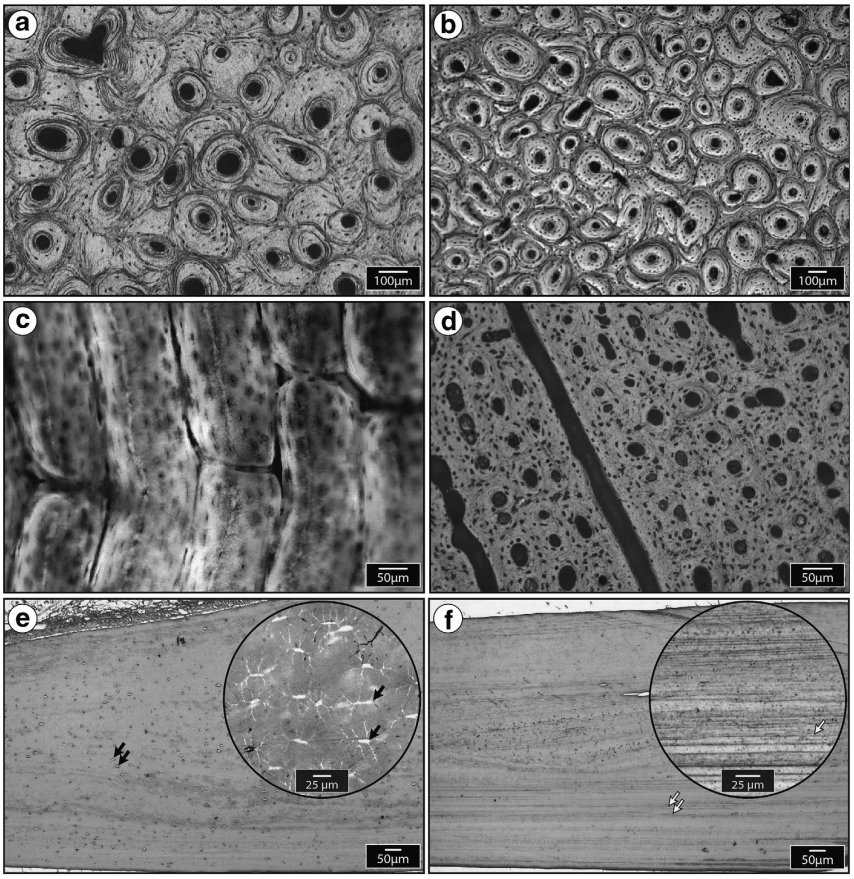

Figure 1 Light microscopy images of transverse cross-sections of bones of representative species from different families of vertebrates. Note the different bone gross structural arrangements across the diverse species and, in particular, the high densities of osteocytes (small black dots, ~15 µm) in (**a**–**d**) relative to the lower osteocytes density in (**e**) and lack of osteocytes in (**f**). (**a**) Human (*Homo sapiens*) bone. (**b**) Equine (*Equus caballus*) metacarpal bone. (**c**) Alligator (*Alligator mississippiensis*) femur (fibrolamellar arrangement). (**d**) Chicken (*Gallus gallus domesticus*) femur. (**e**) Carp (*Cyprinus carpio*) 'cellular' opercular bone, inset (a zoom taken from an orthogonal plane) shows lacunae and canaliculi; black arrows indicate osteocytes/lacunae. (**f**) Tilapia (*Oreochromis aureus*) acellular bone. Note complete absence of lacunae. White arrows indicate the layered structure characteristic of this bone.

from a haematopoietic cell lineage, although these cells are somewhat different from their mammalian counterparts. For instance, most osteoclasts in advanced teleosts are mononuclear (although multinucleate osteoclasts do exist), whereas multinuclearity is considered to be one of the defining features of mammalian osteoclasts.[29] In fact, the lack of osteocytes (as well as the lacunocanalicular spaces) is the only fundamental difference between the bones of the majority of teleost fish species (particularly the more derived ones) and those of all tetrapods.[26,30–33] The fact that there are many extant taxa of osteocytic ('cellular') boned fishes, offers a fantastic palette for comparing the role of bone cells in acellular and cellular bone, but also for investigating the effects of osteocyte loss on a phylogenetic scale (especially since acellularity evolved independently several times within fishes).[34]

The evolution of acellular bone from cellular-boned ancestors, and the dominance of this character in advanced teleosts raise the intriguing possibility that a lack of osteocytes brings some functional advantage. To date such an advantage has not been demonstrated, but the huge variety of fishes with different skeletal types, evolutionary histories and ecologies provide a wealth of natural comparisons for the study of bone evolution and biology. In particular, as fishes were the first animals to possess a bony skeleton, it follows that a thorough understanding of the biology of fish bones may provide valuable insight into pathological conditions involving osteocyte death and bone necrosis (for example, Caisson's disease, radiooosteonecrosis and estrogen deficiency[35,36]) and other fundamental issues and open questions currently occupying researchers of bone biology.

Osteocyte biology is complex and there remain many uncertainties regarding osteocyte function, as well as their mechanisms of communication with other cells and their effects on them. Here, we focus our short discussion specifically on three osteocyte functions that have roles in bone's material properties and response to load, and the evidence for these in acellular fish skeletons. A very small number of previously published studies have focused on cell function in fish bone; we synthesize these published investigations to create an emergent picture of fish skeletal biology with regards to modeling, remodeling and mineral metabolism, while drawing on some of our most recent data to support these ideas and delineate avenues for future work.

Modeling: Evidence for and Implications in Acellular Bone

The process of modeling allows bones to respond adequately to changes of load magnitude and direction that may result from changing circumstances such as increased or decreased levels of activity. Bones can adapt their mechanical performance to these changing loading circumstances by changing their external morphology (for example Bonewald,[1] Adachi *et al*.,[37] Burr *et al*.[38] and Chen *et al*.[39]). Modeling involves the addition or removal of bone material via osteoclastic and/or osteoblastic activity on bone surfaces, and is believed to be triggered by osteocytes sensing the need for modeling from the changed strain distribution within the bone material in the altered loading regime. Modeling therefore results in a net increase or loss of bone material, and is thus important in bone development, bone growth and bone adaptation to changing loads.[4,6,40]

Many studies have concluded that fish bone responds to increased loading by the process of modeling; however, most of these were conducted using fish with cellular bones (for example, Deschamps *et al*.,[41] Eissa *et al*.,[42] Fiaz *et al*.[43] and Totland *et al*.[44]). The few studies that have focused on acellular-boned fishes have provided somewhat secondary evidence for modeling. For instance, Kranenbarg *et al.*,[45,46] described the formation of an abnormal ventral curvature of the vertebral column of sea bass (*Dicentrarchus labrax*) when they were exposed to increased swimming activity, and speculated that the change occurred as an adaptation to increased bending moments caused by the axial musculature. Similarly, Kilhara *et al.*[47] showed that lordosis can be induced in juvenile red sea bream *(Pragus major*) by increased swimming activity. Bone modeling was also demonstrated by Meyer,[48] Huysseune *et al.*,[49] Hegrenes,[50] Witten and Huysseune,[33] and Muschick *et al.*[51] who showed adaptive changes in the pharyngeal jaw bones of cichlids (*Astatoreochromis alluaudi* and *Amphilophus spp.*) in the form of gross reshaping of the jaws and thickening/reorientation of internal trabeculae, in response to increased mechanical load caused by being fed a harder diet.

Although these studies indicate gross changes in bone shape in response to environmental influences, they do not clearly demonstrate at a fundamental level the direct association between loading and modeling. One possible experimental approach to resolve some of these issues is to apply controlled loads to acellular bones and compare their adaptive responses with those of similarly loaded cellular bones. In a study we are currently conducting, we developed an *in vivo* loading model where acellular bone formation is stimulated by the application of loads, controlled in terms of magnitude, direction and duration. In this study, the opercula of both cellular and acellular fish (see **Figure 2**) are loaded using orthodontic 9-mm Nickel-titanium alloy (NiTi) springs, attached to the bone with self-tapping orthopedic screws and applying a constant compressive force of 2 N to the bone material between the screws. The static load generated by the spring is superimposed on the physiological load acting on the operculum, creating a supra-physiological dynamic load shown to induce modeling response in tetrapods.[52] Concurrent with loading, fluorescent labeling dyes are administered via intraperitoneal injections at defined time points during the experiment, allowing measurement of mineral apposition rates in loaded and unloaded areas of the opercular bone.[27,53]

Preliminary results suggest that both cellular and acellular fish bones respond to these mechanical loading regimes. Specifically, uneven bone formation can be observed: the mineral apposition rate in the 'loaded' area, located between the screws, is noticeably greater than the mineral apposition rates in the 'unloaded' area adjacent to it (which is only subjected to normal physiological loads; see **Figure 2**). These results were essentially similar in both cellular- and acellular-boned species, although the mineral apposition rate was higher in the cellular bone in both the unloaded as well as the loaded areas. These preliminary results suggest that indeed, acellular bone does detect and is able to respond to mechanical stimulation. A much larger study is currently under way. More such controlled and quantitative studies are necessary to determine whether and how acellular bone responds to increased loading by increasing its bulk, as well as to assess the effects of loading on the mechanical properties of the newly formed bone material and on its structure at various length scales.

Regulatory pathways in bone are complex, multidirectional and multifaceted, with new (and often paradigm shifting)

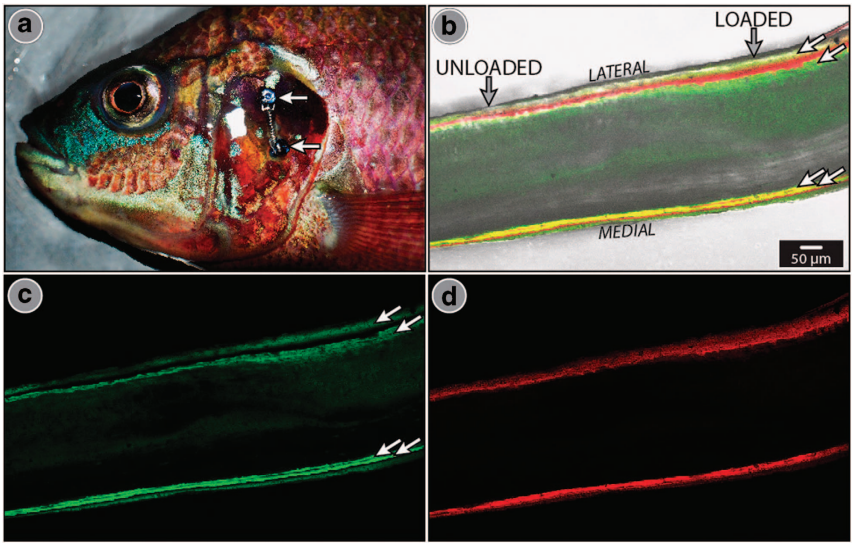

Figure 2 Outcome of three intraperitoneal injections of fluorochrome dyes in tilapia operculum loaded by orthodontic springs. The injections were made 1 week apart (day 0-calcein, day 7-alizarin red, day 14-calcein). Opercula were loaded by fixing a 9-mm NiTi 2 N spring between two self-tapping screws (12 mm apart; indicated, by white arrows, on the operculum of a tilapia in (**a**)). In cross-sections taken perpendicular to the spring connecting the two screws, opercula show differential growth in 'loaded' versus 'unloaded' regions of the outer (lateral) opercular tissue, suggesting load response. Note thicker layer of mineralization in the right top corner of the cross section in (**b**), an overlay of calcein (green fluorescence) and alizarin (red fluorescence) staining images. Data from the separate stains are shown individually in (**c**) and (**d**), respectively; white arrows indicate the two separate injections of calcein.

perspectives gained regularly (for example, Bonewald,[1] Kennedy and Schaffler,[3] Baron *et al*.,[54] Lu *et al*.,[55] Martin *et al*.,[56] Nakashima *et al*.[57] and Xiong *et al*.[58]). It is beyond the scope of this review to discuss the complete range of known and hypothesized osteocyte factors and mechanisms for regulating modeling and remodeling, especially as many of these have yet to be investigated in fish bone, both cellular and acellular. We, however, highlight two signaling proteins of primary importance in mammalian bone—sclerostin and receptor activator of nuclear factor-κB ligand (RANKL)—which demonstrate the extent to which osteocytes are involved in formation and resorption of bone material.

Sclerostin (SCL), a protein encoded by the *SOST* gene, is secreted almost exclusively by osteocytes and is capable of suppressing bone formation.[54,58,59] When bone is subjected to loading, *SOST* expression and sclerostin levels decrease significantly, stimulating osteoblast activity and probably (through an effect on RANKL) inhibiting osteoclasts.[1,60,61] On the other hand, osteocytes have also recently been shown *in vivo* and *in vitro* to be the primary expressers of RANKL, a regulator of osteoclast differentiation and activation.[57,58,62,63] RANKL is upregulated (resulting in targeted tissue removal) when osteocytes are damaged, die by apoptosis or experience prolonged loss of mechanical loading, and recent evidence also suggest that osteocyte RANKL may be specifically involved in the remodeling process of cancellous bone.[58,64,65]

Given that signaling proteins like sclerostin and RANKL allow osteocytes to act as significant regulators of osteoblast and osteoclast activity and the interplay between them, controlling the rate and extent of bone formation and resorption, it is appealing to ask what control mechanisms exist in bone without osteocytes, and which mechanisms may be conserved between fishes and other vertebrates. Although these questions are largely unaddressed in fishes (but see To *et al*.[66]), it is clear that, in general, the regulatory measures attributed to osteocytes are not simply absent in acellular bone. Moreover, acellular bone does not appear as mammalian bone with the osteocytes simply removed or dead, a manipulation that alters SCL and RANKL levels and can have drastic effects on the balance between bone gain and loss. For example, deletion or mutation of the *SOST* gene, which is responsible for SCL production in mammals, results in excessive bone mass (for example, sclerosteosis[16,54]), and the loss of osteocytes via apoptosis or fatigue damage in mammals has been shown to be linked to increase in osteoclast activity.[22,36,65] In a mouse model where osteocytes were experimentally ablated, osteoclast number and activity increased markedly, reducing bone material quality.[18]

Clearly, although osteocytes are lacking, bone production and removal do not run rampant in acellular fish bone, although RANKL has recently been shown to exist and have a conserved role in the skeleton of an acellular teleost fish.[66] This suggests alternative regulation factors and/or control pathways, perhaps via osteoblast control of RANKL, although parathyroid hormone (known to promote osteoblast expression of RANKL in mammals) is present in quite different form in fishes, and fishes lack parathyroid glands.[60,67]

Determination of the precise triggering and activation machineries involved, the sequence of gene expression in regulation and coordination, and the cells that take part in sensing the need for fish bone modeling can be made by a combination of histological, biochemical and molecular genetics methods. Even if the cells in the bones of fishes are shown to possess different functions than those in mammalian bone, comparison of these systems will shed invaluable new light on our understanding of bone cell biology, diversity and function. As current therapy concepts for syndromes like osteoporosis assume that osteocytes orchestrate bone formation and adaptation,[60] results of the proposed investigations may also open the possibility to design new strategies for clinical treatment.

Remodeling: Evidence for and Implications in Acellular Bone

Remodeling is another fundamental, osteocyte-mediated bone capability, which allows the tissue to respond to challenging environmental circumstances.[6,64,68] As in other materials experiencing prolonged and repeated use, tetrapod bones often accumulate fatigue damage in the form of microcracks.[69,70] Remodeling allows bones to replace these damaged regions within their bulk with new bone material, via coordination of the activity of osteoblasts and osteoclasts. This dynamic remodeling process is triggered by osteocytes.[6,64,68]

It could be argued that remodeling would be unnecessary in fish bone; whereas the forces affecting tetrapod bones are created by a combination of gravitational forces and muscle forces, the loads applied to the fish skeleton result almost exclusively from muscle contractions. However, i*n vivo* measurements of strains in the opercular bone of the bluegill sunfish *Lepomis macrochirus* showed that peak strains reached 2000 microstrain, similar to peak strains measured in bones from a variety of mammals and birds, suggesting that fish bone operates under performance demands similar to those in tetrapod bone that result in remodeling.[71]

Remodeling has never been explicitly demonstrated in acellular fish bone, although some anatomical studies exhibit tissue morphology suggesting the process (see below), and both modeling and remodeling processes could be involved in the diet-induced morphological changes to jaw tissue mentioned above. The importance of remodeling for tetrapod bone suggests such maintenance mechanisms would hold similar advantages to fish bone as well. In fact, the extreme ecologies, high performance levels and long life spans of some acellular fish species lead us to speculate that similar mechanisms may be indispensable to them. Lauder and Lanyon's[71] *in vivo* measurements of surface strains from bluegill sunfish showed that not only were peak strains similar across taxa, the strain rates in fish bone were an order of magnitude higher than those considered normal in mammals. If, on the other hand, remodeling is not occurring to meet this demand, this raises the possibility that the structure and internal architecture of acellular fish bone is damage-resistant, at least to such an extent that only minor amounts of damage accumulate so that remodeling is not required. A material with such properties could inspire novel and mechanically useful biomimetic materials, and begs a thorough and detailed study of the hierarchical structural elements, architecture and mechanics of acellular fish bone.

In order to examine the possibility of remodeling in acellular-boned fish, we turned to a family of fishes characterized by regular, extreme loading scenarios that would make remodeling critical. The billfishes (Xiphiidae and Istiophoridae) have long lives (up to several decades), and can reach large body sizes and high swimming speeds (some species reaching 600 kg and 80 km h^{-1}, respectively), but are most recognizable by the large pointed rostra or 'bills' from which they get their common name. The bills are sword- or spear-shaped and comprise elongated cranial bones, in some species reaching impressive lengths (up to 1/3 of the entire length of the fish). The bills may be used for hunting, defense and/or drag reduction, and so are likely heavily and cyclically loaded in cantilever bending over the fishes' long lifespans; they therefore represent an extreme example of acellular bone surely in need of regular remodeling to avoid failure.

In collaboration with Philip Motta and Laura Habegger at the University of South Florida, who study the mechanics of the whole bill, we are currently conducting a study of the structure, composition and mechanical properties of the bone material of the bills of five species of billfishes, which vary in body size and relative bill length. In our preliminary anatomical evaluations of the rostral aspect of the bill of these five species (an area likely to experience particularly high stresses and strains), we consistently saw numerous features resembling secondary osteons, structures considered definitive evidence for remodeling in tetrapods (**Figure 3a**). As in tetrapods, the 'osteons' we observe in billfish bone are visibly distinct structures, comprising concentric layers of bone surrounding hollow tubes (likely containing blood vessels, as they do in tetrapods). Using backscattered scanning electron microscopy, we show that this bone tissue consists regions of graded mineral density (**Figure 3b**). Polarized light microscopy shows the frequent presence of 'osteons' overlapping or 'cutting into' one another (**Figure 3c**); such 'secondary osteons' are characteristic of remodeling in tetrapod bone, morphological indications of the processes of removal of previously existing material and its replacement with new bone. Billfish secondary osteonal morphologies are so characteristic as to fool any bone biologist into thinking they were looking at remodeled mammalian osteonal bone, but for the fact that the tissue, rather than being rife with osteocytes as in tetrapods, is completely devoid of cells (compare **Figures 3b–c**).

Although Moss and Freilich[72] stated that osteonal bone is rare in fishes and only found in larger species, osteonal morphology similar to that seen in billfishes has since been observed for species of a variety of sizes: two cellular-boned ostariophysian fishes (*Myleus ternetzi* and *Sciades proops*; see Figures 10 and 11 in Meunier *et al.*[73]) and two acellular-boned acanthopterygian fish (*Trachurus mediterraneus* and *Xiphias gladius*[74,75] and our study), with secondary (overlapping) osteons clearly visible. These observations suggest not only that acellular and cellular osteonal bone may be common in fishes (the species listed above are from a range of phylogenetic groups) but also that acellular bone indeed has the ability to remodel.

Further studies are needed to determine whether microdamage occurs and accumulates in fish bone, and what the triggering mechanism is for the observed modeling and remodeling in the absence of osteocytes. The proposed hypothesis,[33] that osteoblasts are involved in mechanosensing in acellular bone, is supported by Kitamura *et al.*'s[76] demonstration in the (acellular and dermal) scales of goldfish of increased osteoblast but not osteoclast activity (via alkaline phosphatase (ALP) and tartarate resistant acid phosphatase (TRAP) staining, respectively) in response to increased swimming activity, and by *in vitro* studies of mammalian mesenchymal tissue-derived cells at various stages of differentiation showing response to their mechanical environment.[77–81] Lu *et al.*'s[55] illustration *in vitro* of higher mechanosensitivity in mammalian osteocytes than osteoblasts, however, suggests perhaps a different level of osteoblast mechanosensitivity in fish bone, starkly different loading demands, bone material properties in fishes and mammals, and/or recruitment of other mechanosensors in addition to

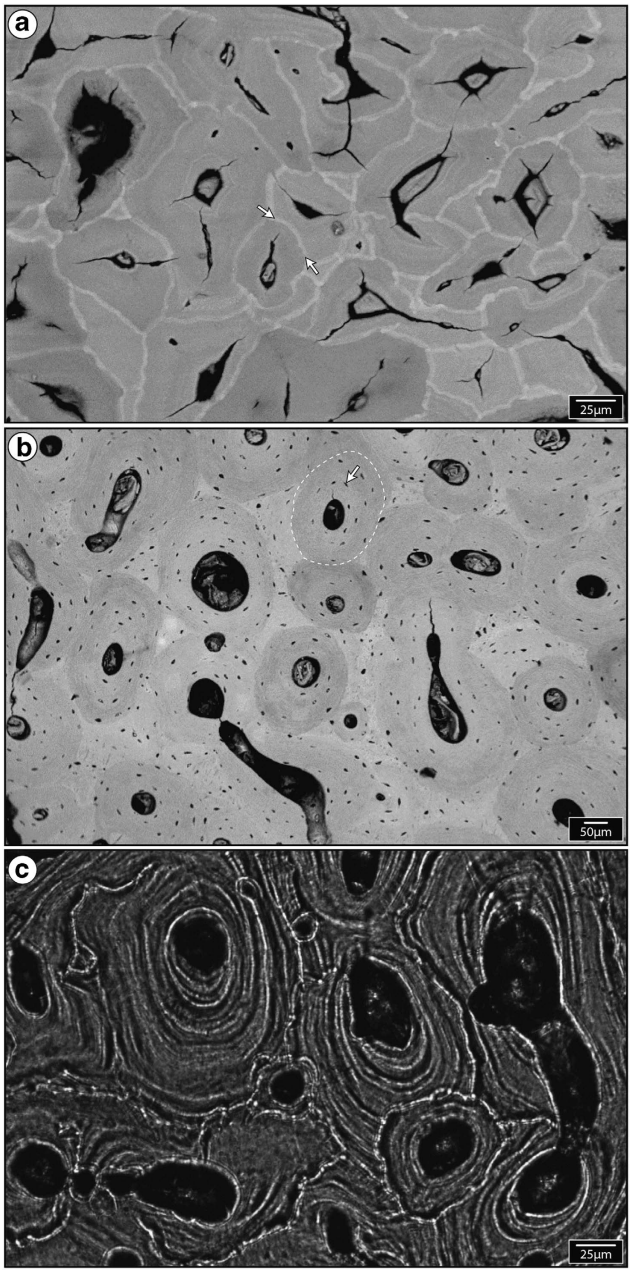

Figure 3 Light and electron microscopy images of cross-sections of the rostral aspect of the bill of the acellular-boned blue marlin (*Makaira nigricans*), showing morphology similar to mammalian osteonal bone and suggesting remodeling. (**a**) 'secondary osteons' in a transverse section of the bill of a mature blue marlin. White arrows indicate the 'cement line' of one such 'osteon' cutting into another 'osteon'. Note complete absence of osteocytes. (**b**) Secondary osteons seen in a transverse section of the equine third metacarpal bone. Note abundance of secondary osteons and numerous osteocytic lacunae (white arrows) and osteonal cement line (dotted line). (**c**) Polarized light microscopy of a blue marlin section (similar to (**a**)), showing the ring-like arrangement of the collagen fibers in the osteons, with obvious remodeling.

osteoblasts. Further studies of osteoblast and osteoclast activity, through *in vivo* performance and selective staining studies, examination of signaling pathways, and *in vitro* cell culture and mechanostimulation work, will help to clarify the roles of these cells in fish bone mechanobiology.

Mineral Metabolism: Evidence for and Implications in Acellular Bone

A third role for osteocytes, which was proposed already over 100 years ago but remains hotly debated, is known as osteocytic osteolysis and relates to the involvement of osteocytes in calcium homeostasis, a key feature of vertebrate metabolism. Extracellular and intracellular calcium levels are extremely tightly controlled by elaborate physiological pathways.[82] The skeleton serves as a major reservoir of calcium, which osteoclasts can access when calcium plasma levels decrease below a given threshold, by resorbing bone and releasing free calcium ions into the circulation. Conversely, when high calcium levels are encountered, calcium is deposited into the skeleton for storage.

Osteocytes have indirect control over the liberation of calcium via their RANKL-mediated initiation of osteoclast proliferation and activity;[57,58,63] upregulation of osteoclast-mediated bone resorption is triggered by RANKL production from both healthy and dying osteocytes (for example, in response to mechanical tissue damage).[22,65] The theory of osteocytic osteolysis, however, proposes more local and direct osteocytic control of the maintenance of adequate serum calcium levels, proposing that osteocytes are also involved in calcium metabolism by dissolving (and possibly reforming) the walls of their lacunae (for example, Bonewald,[1] Bélanger[83] and Teti *et al.*[84]). Osteocytes could be extremely efficient in this function, given the huge advantage in access to bone tissue afforded by their high density and the resulting large surface area of the osteocytic network. There is growing evidence supporting the involvement of osteocytes in the regulation of calcium and phosphate levels, not only indirectly by producing proteins like sclerostin and RANKL, but also directly by molecules that regulate mineral ion availability, such as phosphate-regulating neutral endopeptidase on chromosome X, dentin matrix protein 1, matrix extracellular phosphoglycoprotein and fibroblast growth factor 23. Although osteocytes produce all of these factors, the mode and site (local or remote) of action of each is different, underlining osteocytes' range of effects.[3]

Osteocytic osteolysis was initially supported by many reports that documented increased lacunar volume at times of calcium need, such as during lactation or in pathological conditions such as secondary renal hyperparathyroidism.[83,85] The concept fell out of favor in the 1970s, because of the demonstrated ability of osteoclasts to efficiently resorb bone and technical flaws shown in the technique of measurement of lacunar volume, raising the possibility that the observed increase in lacunar size may have simply been an artifact. However, osteocytic osteolysis is regaining support lately,[1,2] owing to new evidence based on advanced measuring techniques, such as backscatter electron microscopy and acid-etched scanning electron microscopy, allowing more precise demonstration of lacunar enlargement.[86]

Obviously, osteocytic osteolysis is not available to the advanced teleosts, as they lack osteocytes. In fact, acellular-boned fishes have been shown to experience difficulties in mobilizing calcium in times of artificially created need.[87] For instance, when fracture healing was evaluated under conditions of calcium deprivation from the water, it was demonstrated that whereas fish with cellular bone (*Carasius auratus*) formed a mineralized fracture callus, fish with acellular bone

(*Sarotherodon*) could not mineralize the callus they formed.[88] Fortunately, fish rarely encounter this circumstance, as calcium is plentiful in both sea and fresh water, and thus is a readily available reservoir during times of demand.[33] It was furthermore demonstrated that if calcium deficiency is imposed on fishes artificially, they will preferentially mobilize calcium stores by resorbing their scales (exoskeleton) rather than the bones of the endoskeleton.[87] In fact, it has been shown that the more common cause for bone resorption in fish is phosphorous deficiency.[33,89,90] Phosphorus is an essential mineral for fish, needed for bone mineralization as well as for various metabolic pathways. However, as opposed to calcium, its availability in both fresh water and seawater is relatively low, and adequate levels depend on dietary intake.[89] Phosphorus deficiency leads to improper skeletal mineralization and skeletal abnormalities.[89] In particular, it has been shown that in at least one species of acellular-boned fish (haddock, *Melanogrammus aeglefinus*) phosphorous deficiency initially led to increased bone resorption, and subsequently to decreased bone mineralization and formation.[91] Future studies should aim to compare the response with phosphorous deficiency between cellular- and acellular-bone fish. We hypothesize that osteocytes in cellular-boned fish may partake in bone resorption in times of phosphorous need by a process akin to osteocytic osteolysis, whereas acellular-boned fish would be more sensitive and have more difficulty in adequately responding to such environmental circumstances.

Conclusions

Considering the very large number of extant fish species and the vast and detailed literature describing the bones of other vertebrates, it is surprising that so little is known about the biology, architecture and material properties of fish bone, in particular the types that lack osteocytes. The possibility that bone can 'sense' and respond to the need for adaptation and tissue replacement, and regulate these processes without these cells must mean that alternative mechano- and homeostatic sensing mechanisms are present, or that these processes are somehow less important in fishes. The existence of acellular bone therefore provides a common and naturally occurring model to critically examine the possibility that osteocytes are not the sole regulators of modeling and remodeling of bone, with an obvious first step being to determine just how widespread these processes are in fishes.

We suggest that all of the important osteocytic functions we detail above—bone modeling, remodeling and mineral homeostasis—are also occurring in acellular fish bone and so are clearly carried out through alternative pathways. One may speculate that because calcium deficiency in fishes is rare and rather unlikely to occur (rendering the need for osteocytic osteolysis obsolete), and because modeling and remodeling still appear to occur, the metabolic cost of maintaining osteocytes within the bony matrix became unnecessary, leading to an evolutionary pressure toward acellularity. We can only understand the selective pressures leading to the loss of osteocytes in fishes (or the factors maintaining their presence in cellular species) through determination of the roles had by the cells in fish bone. In addition to projects focused on characterization of the osteocytes of cellular boned fishes and comparison with tetrapod osteocytes, comparison of osteoblasts and osteoclasts among fish species—particularly in those groups with both cellular- and acellular-boned species[45]—will clarify whether cellular functions are conserved among species and groups or are more variable than previously appreciated. Such studies may also help us to approach mammalian skeletal biology from new perspectives, for example through re-evaluation of the importance of mono-nucleated bone-resorbing cells and the determination of non-osteocytic mechanosensors.[29] Furthermore, projects aimed at understanding the mechanobiology of fish bone (for example, the agents of mechanosensation in acellular bone, the nature and frequency of microdamage, the mechanisms of tissue repair and modeling) will surely provide insight into the loading demands of fish skeletons and the evolution of mechanosensation, but also the mechanical performance of a skeletal biomaterial lacking components impossible to manufacture in manmade composites, the cells.

Conflict of Interest

The authors declare no conflict of interest.

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