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Review

Fine structure of bone matrix calcification

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ABSTRACT

Various functions of bones are derived from the characteristics that they calcify. In bone calcification, hydroxyapatite is crystallized on the type I collagen-based organic matrices. The organic components of those bone matrices are commonly shared with other fibrous tissues. As extracellular fluid supersaturates hydroxyapatite, the reasons why bones are specifically calcified remains enigmatic. With the bone matrix calcification, the structural and the spatial changes of those organic substances seem to be closely associated, and participate in the complicated regulation of hydroxyapatite multiplication. The present paper overviews the morphological findings of bone calcification, and discusses the mineral and organic environments in bone matrix calcification.

Contents

1. Introduction

Bone is a typical example of hard tissue. The bones work to shape, support, and protect body structures. They also aid body movement, house bone marrow, and store various minerals essential for the preservation of life. All these functions are derived from the characteristics that the bones calcify. The hardness of bones is derived from the specific composition. Compared with in soft tissues, large part of water is replaced by inorganic materials in bones. Calcium (Ca) and phosphorus (P) are major components of the inorganic compositions of human bones. The Ca/P ratio indicates high value of 2.33. It suggests that crystals in bones almost consist in hydroxyapatite (HA). Bones contain not only inorganic materials, but also organic substances, including collagen, proteoglycan, and other non-collagenous matrix proteins [1]. Those components are not unique for bones, but are commonly shared with other fibrous tissues including skin, tendon, or ligaments. It is well known that extracellular fluid supersaturates for forming HA [2]. As the entire body is filled with the fluid containing high concentration of calcium and phosphorus, it is always suffering from pathological calcification. However, fibrous tissues other than bones keep the uncalcified situation in fact. The reasons why only bones are calcified remains enigmatic.

The previous findings from morphological view demonstrated that matrix vesicles start the primary calcification in bones [3]. Osteoblasts bud the 30- to 100-nm-wide matrix vesicles, which are surrounded by a lipid bilayer. Within the matrix vesicles, crystallized HA appears and grows, showing the primary calcification (Fig. 1, top). As those matrix vesicles were found, not only in the bones, but also in cartilage, dentin, or other hard tissues [4,5], the HA crystallization in the matrix vesicles is regarded as the pivotal event for biological calcification.

Otherwise, HA crystals subsequently multiply, forming calcified nodules that expand, fuse, and then calcify expansive matrix

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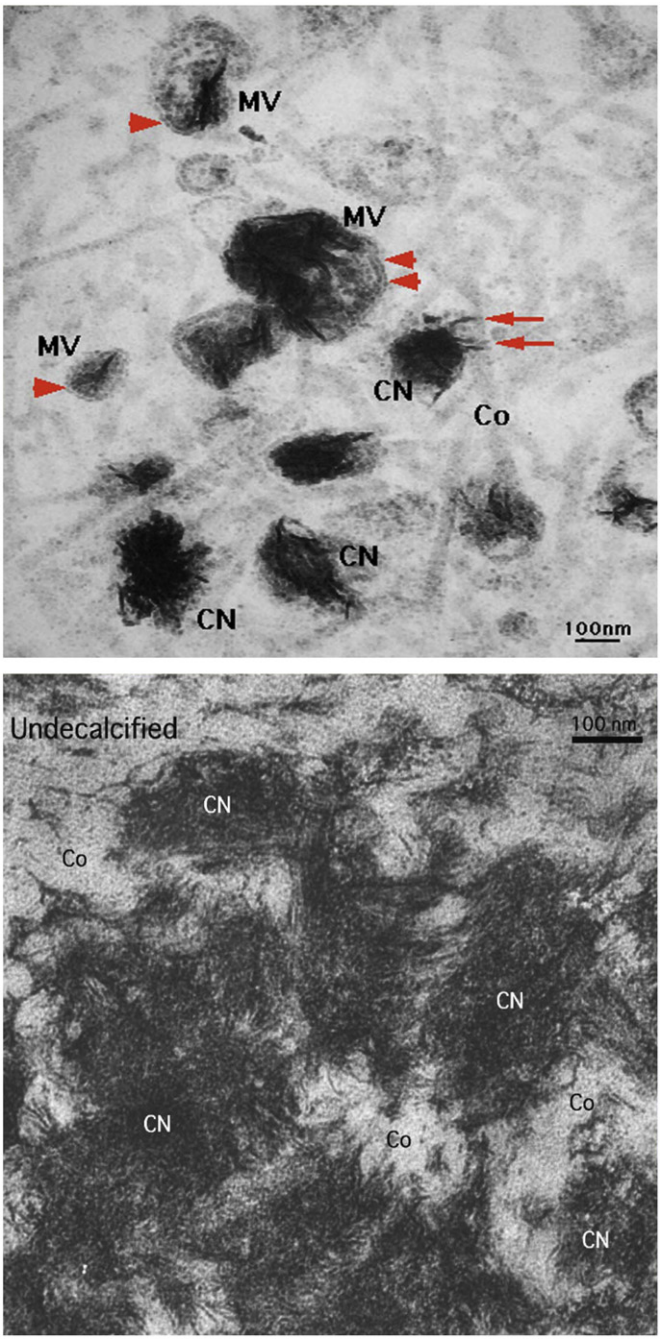

Fig. 1. In the new bone, round osteoblasts and immature calcifying matrix are present. In immature bone matrix, calcification advanced as the distance from osteoblasts increased. Within membrane-bound matrix vesicles (MV), crystallization occurs (top, arrowheads). Subsequently, calcified nodules (CN) formed, followed by focal calcification along collagen fibrils (Co), which are referred to as collagen calcification (top, arrows). Finally, calcified matrix was expanded (bottom). The images were rearranged from Ref. [5].

(Fig. 1, bottom). The expansion of calcification in matrix is another clue to solve the molecular mechanisms of bone calcification. With this process, the structural and the spatial changes of various organic substances in bone matrix seem to be closely associated, and participate in the complicated regulation of HA multiplication. The present paper overviews the morphological findings of bone calcification, and especially focuses on the interaction between matrix molecules and bone minerals in the stage of expansive calcification.

2. Contents

2.1. Formation of calcified nodules

The immature bone matrix, uncalcified osteoid, consists of many organic substances, including proteoglycans, non-collagenous matrix proteins, phospholipids, and alkaline phosphatase (ALP). Those substances can interact with calcium or phosphate, both of which are components of HA. However, those organics can be detected in both bone and soft tissue. Only the presence of those substances in bones cannot explain the mechanisms of bone matrix calcification. More important issue seems their structural and localizational alterations triggered by biological action of osteoblasts.

Those organics interacting with minerals were reported to change their structures and localizations, during the transition from the uncalcified to the calcified phase, which affected the mineral microenvironment and initiate calcification [1]. Energy-filtering transmission electron microscopy (EFTEM) is useful to observe the morphological changes of both molecular structures and mineral localizations. Conventional transmission electron microscopy (TEM) enables us to visualize images through the contrast produced by elastic electron scattering. Electrons that pass through the elements include inelastically scattered types, which lose a measurable amount of energy, after their collision with inner-shell electrons, identified as being the principal causes of the aberration typically encountered in this method. EFTEM, on the other hand, can visualize the distribution of those inelastically scattered electrons that are marked by the loss of varying amounts of energy. By setting energy loss at 0 eV, most of the inelastically scattered electrons are eliminated, thus revealing images of a higher resolution than any obtainable via conventional TEM (Fig. 2, top left). In addition, certain elements are mapped at that particular level, when the electrons exhibiting energy loss typical of the elements are localized. Because the spatial resolution of the conventional electron probe X-ray microanalysis depends on the spot size of the electron beam, it usually is confined to a range between a few micrometers and some hundreds of nanometers. However, as resolution of EFTEM during elemental analysis corresponds to that of conventional TEM, mapping of Ca or P at the molecule level becomes possible. In order to analyze bone matrix calcification, alterations of the mineral microenvironment according to the ultrastructural and localizational changes in organic substances during bone calcification were visualized by the application of EFTEM. When observing the calcifying bone in the intramenbraneous ossification, Ca was localized in certain osteoid and throughout areas of calcification. Similarly, P was localized in some osteoid and all calcified areas. However, their localization patterns in the osteoid were not the same. Under higher magnification, a calcified nodule, collagen fibrils, or proteoglycan meshworks among them were clearly observed by EFTEM (Fig. 2, top), and Ca and P also colocalized in calcified nodules (Fig. 2, bottom). However, the Ca of osteoid tended to localize at proteoglycan meshwork among collagen fibrils (Fig. 2, bottom left), whereas P often maps to collagen fibril structures (Fig. 2, bottom right). Ca and P were preserved in different areas of uncalcified matrix, while both colocalize after the formation of calcified areas.

In order to discuss the mineral localization in and around the calcified nodules, the organics interacting with minerals were localized at TEM level [1]. Progeoglycan, possessing high affinity with Ca, such as decorin and chondroitin sulfate, are immunohistochemically found along the osteoid at light microscopic level, while Cuprolinic blue stain can enhance proteoglycans in TEM images. CB-based TEM observation highlighted the fact that positively stained structures tended to be smaller the further they