PERSPECTIVE

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The material composition of bone – A brief overview of old, new, and emerging concepts

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ABSTRACT

Bone is a complex biological system where the interplay between composition and structure governs the overall function. In addition to the constituents of the mineralised compartment (i.e., the extracellular matrix), the unmineralized compartment comprises vascular spaces, the osteocyte lacuno-canalicular network, and nanoporosities that contain water. Dehydration causes embrittlement. The mineralised collagen fibril is the basic unit of structure and function. Recent findings reveal that micrometre-sized marquise-shaped motifs bridge the length scales between individual mineralised collagen fibrils and the interwoven mesh-like architecture. With little evidence of hydroxyl (OH^-) groups, the bone mineral must be viewed as carbonated apatite with various ion substitutions such as CO_3^{2-}, Mg^{2+}, and HPO_4^{2-}, rather than as hydroxy(l)apatite. This mineral is found both around the fibril (i.e., extra-fibrillar mineral) and within the fibril (i.e., intrafibrillar mineral). Finally, the extra-fibrillar mineral takes the form of curved, polycrystalline lamellae that closely wrap around individual collagen fibrils in addition to uncurved (flat) lamellae.

Keywords: Apatite, Bone, Biomineralization, Biomaterials, Bone-Implant Interface

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INTRODUCTION

The organic and the inorganic

Of all the materials known to man, whether naturally-occurring or synthetic, bone is arguably the most intriguing nanocomposite material – engineered by Nature. Bone is tough and built to withstand. Moreover, flaws can be repaired and areas of microdamage are replaced by pristine tissue through a programmed and highly intricate process of remodelling. The fundamental *material* properties of bone are derived from the extracellular matrix whose two major constituents are type-I collagen and biological apatite. A minor fraction of non-collagenous proteins also exists and serves important regulatory and biological functions. Beyond the chemical composition on the material level, bone also exhibits a *hierarchical* architecture where large structures are composed of progressively smaller building blocks – down to the atomic level. Both compact and spongy bones are made

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up of lamellae, which are in turn made up of mineralised collagen fibrils where the organic and inorganic components occupy mutually reciprocal spaces.

While the osteocytes are terminally differentiated cells housed within small elliptical cavities called lacunae that are peppered throughout the mineralised matrix **(Fig. 1a)**, the osteoblasts are exclusively confined to the surface of the bone. Buenzli and Sims have estimated that the average adult human skeleton is home to ~42 billion osteocytes.[1] However, this does not entirely reflect the even higher number of bone-forming cells, i.e., the osteoblasts, required to produce the skeleton. Empirical, densitometric investigations of Pazzaglia and co-workers indicate that only one in every ~67 osteoblasts slow down matrix production and consequently become entrapped within the surrounding mineralised matrix.[2] However, certain vertebrate species (i.e., some types of fish) possess bone that does not contain osteocytes embedded within the mineralised matrix.[3] The obvious implication is that the process of bone formation is not identical across all species. Are there parallels to be drawn between acellular bone and other mineralised tissues such as dentine, which only houses the cytoplasmic extensions of odontoblasts but not cell bodies, or the dental enamel, which is entirely devoid of ameloblasts?

Water, water, everywhere

Water is the third but not the least important component of bone. Broadly, water exists in two major compartments. The first is water that resides freely within the vascular and the lacuno-canalicular spaces and can flow according to pressure gradients that develop during the movement of the skeleton. The second is water that is bound to the extracellular matrix and is distributed across three sub-compartments. These are either (*i*) *loosely bound water*, which refers to water found at the surface of the collagen fibrils and between the collagen and the mineral phase, (*ii*) *tightly bound water*, which refers to water molecules trapped inside the collagen triple helix, and (*iii*) *structural water*, which refers to the water molecules found within the core of the apatite structure.[4] Indeed, water is a significant contributor to the eventual mechanical competence of bone, as has been demonstrated experimentally that dehydration, either by air-drying or by use of certain solvents, causes the bone to become brittle.

The mineralised collagen fibril is the basic unit of structure and function

On the nanoscale, collagen fibrils are made up of tropocollagen molecules that are approximately 1.5 nm in diameter and 300 nm in length **(Fig. 1b)**. More precisely, an individual collagen fibril (< 100 nm in diameter) is assembled from five tropocollagen molecules that are arranged in parallel but remain staggered with respect to each other by ~67 nm. This peculiar offset distance is approximately one-fourth the length of the tropocollagen molecule. Therefore the stacking arrangement of tropocollagen molecules within the fibril is referred to as 'quarter-staggered'[5], giving rise to the characteristic cross-striated pattern **(Fig. 1c-d)** The mineral is found both within the fibril (i.e., intrafibrillar mineral) and around the fibril (i.e., extra-fibrillar mineral). This unit of structure and function is collectively referred to as the 'mineralised collagen fibril'. In both lamellar (ordered) bone and woven (disordered) bone, the mineralised collagen fibril is the main building block of the extracellular matrix. Bundles of mineralised collagen fibrils give rise to *twisted* rope-like fibres that in turn form an interwoven mesh-like architecture **(Fig. 1e-f).**[6] The mechanism that achieves these rope-like fibres, that continuously run almost uninterrupted for tens of micrometres, remains shrouded in mystery. Though it may be speculated that synchronised clusters of osteoblasts function in a highly coordinated manner to actively align individual collagen fibrils into the eventual rope-like fibres.[7]

Closer examination reveals an intermediate hierarchical level of organisation between the individual mineralised collagen fibril level and the rope-like fibre level. Bridging the gap between the two length scales are micrometre-sized *marquise-shaped* motifs **(Fig. 1g-h).**[8] In the transverse view, these features appear round and have been referred to as *rosettes* **(Fig. 1i)**, however, these structural units are more accurately described as *prolate ellipsoids* when viewed in 3D.[9] On the nanoscale, mineralised collagen fibrils can be observed using highly sophisticated analytical techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Not only normal (or otherwise disease-free and healthy) bone, but also the extracellular matrix of bone exposed to antiresorptive agents such as bisphosphonates[10], the bone around osseointegrated metal implants **(Fig. 1j)**[11], and bone

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formed in response to bioactive materials such as multi-component calcium phosphates (CaP) **(Fig. 1k)**[12] displays a remarkably similar nano-to-micron scale organisation of mineralised collagen fibrils. However, certain conditions such as X-ray irradiation can cause alterations in collagen secondary structure. Some of these changes are detectable using vibrational spectroscopy techniques such as Raman spectroscopy and Fourier transform infrared spectroscopy, and include increased crosslinking and breakage of peptide bonds in the collagen backbone with embrittlement of bone due to degradation of the collagen matrix.[13]

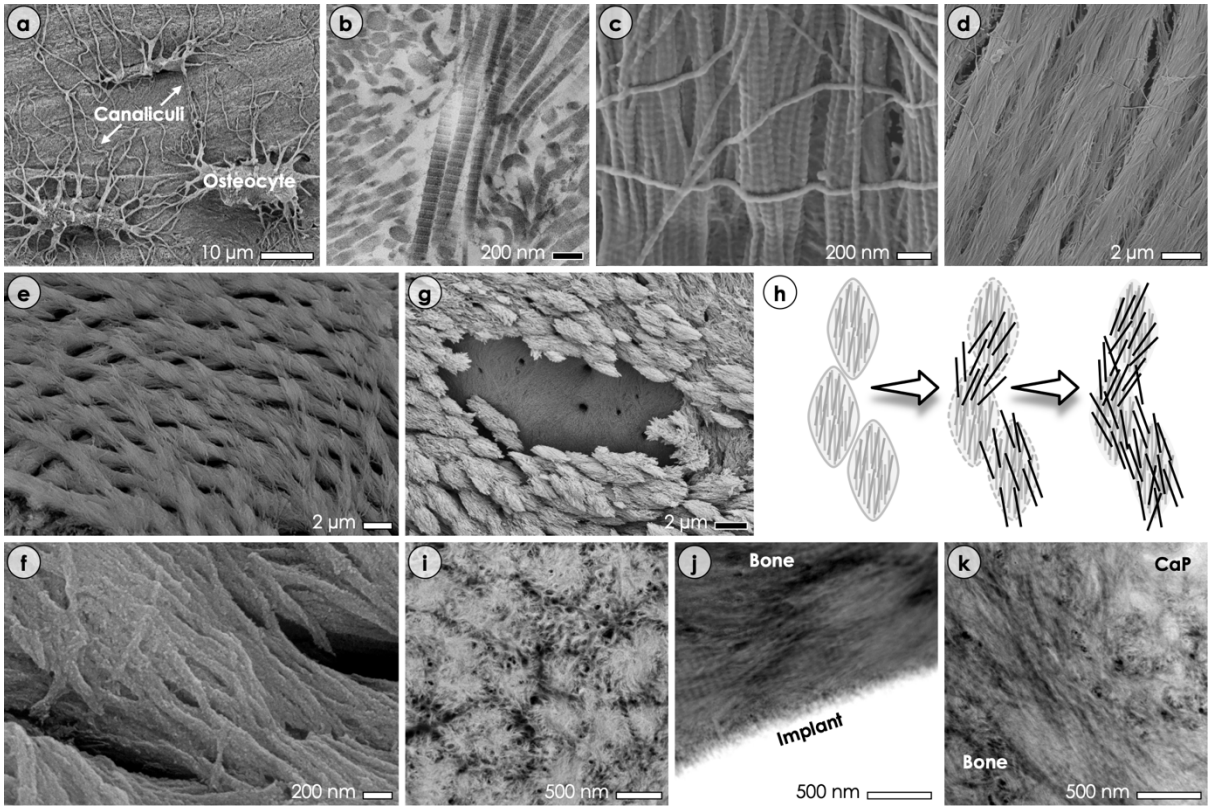

Fig. 1. (a) The osteocyte lacuno-canalicular network in bone observed using the resin cast etching technique (SEM). (b) Collagen fibrils in decalcified bone (TEM). (c-d) Collagen fibrils in a partially decalcified bone at high- and low magnifications, respectively (SEM). (e-f) The interwoven mesh-like architecture of mineralised collagen fibrils at low- and high magnifications, respectively (SEM). From Shah FA et al. Calcif Tissue Int. 2016[6]. (g) Marquise-shaped motifs observed at the bone surface (SEM). From Shah FA et al. Bone Rep. 2020[8]. (h) Illustration of transformation of bone mineral morphology from discrete marquise-shaped motifs to the interwoven mesh-like architecture. From Shah FA et al. Bone Rep. 2020[8]. (i) Rosettes (TEM). From Shah FA et al. Bioact Mater. 2023[12]. (j) Mineralised collagen fibrils in bone interfacing an osseointegrated metal implant (TEM). From Shah FA et al. Nanomedicine. 2014[11]. (k) Mineralised collagen fibrils in bone interfacing a CaP biomaterial (TEM). From Shah FA et al. Bioact Mater. 2023[12].

Bone mineral is neither hydroxy(l)apatite nor always flat

X-ray diffraction studies reveal that the structure of bone apatite is comparable to hydroxy(l)apatite standards such as the National Institute of Standards and Technology (NIST; SRM 2910), albeit less crystalline. The term hydroxy(l)apatite, however, suggests the presence of hydroxyl (OH^-) groups within the crystal lattice but vibrational spectroscopy confirms that bone mineral does not contain any OH^- groups.[14] Various ionic substitutions exist within apatite of biological origin, including carbonate (CO_3^{2-}) for phosphate (PO_4^{3-}),

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magnesium (Mg^{2+}) for calcium (Ca^{2+}), and small inclusions of sodium (Na^{+}), potassium (K^{+}), fluorine (F^{-}), chlorine (Cl^{-}), zinc (Zn^{2+}), strontium (Sr^{2+}) etc. Other impurities within bioapatite include acid phosphate (HPO_4^{2-}) groups. Therefore, an important aspect of the composition of the bone extracellular matrix that must be reiterated is that the inorganic phase is *not* hydroxy(l)apatite. Instead, the bone mineral must be viewed as ion-substituted carbonated apatite.

CO_3^{2-} ions introduce disorder and reduce mineral crystallinity.[15] The term *crystallinity* describes the degree of structural order in a solid. In a crystal, the arrangement of atoms or molecules is repetitive and consistent. Mg^{2+} ions, on the other hand, despite being well-established inhibitors of hydroxy(l)apatite crystallisation, do not induce phase changes in bone mineral. Although the distorted, high-energy surface of bone apatite crystals accounts for the detection of amorphous calcium phosphate[14], there is no evidence for the occurrence of other types of biominerals within the extracellular matrix such as magnesium whitlockite [$Ca_{18}Mg_2(HPO_4)_2(PO_4)_{12}$], which is otherwise associated with pathological calcifications.[16] Biomineralisation tends to be a carefully regulated process. The rare occurrence of magnesium whitlockite within osteocyte lacunae, seen as part of the condition called *micropetrosis*, suggests an altered local biochemical environment and represents a complex multifactorial process.[10] A straightforward increase in circulating Mg^{2+} levels is unlikely to induce the formation of magnesium whitlockite in the absence of an appropriate local pH.

For the most part, the extra-fibrillar mineral occurs in the form of thin plate-like structures, referred to as *mineral lamellae*, which are either flat or curved to wrap closely around collagen fibrils.[17] Each mineral lamella is polycrystalline, i.e., made up of multiple crystallites. Within the first few nanometres (< 20 nm) from the collagen fibril, the radius of curvature (of the mineral lamellae) matches that of the fibril diameter. At greater distances, however, mineral lamellae form flatter arcs of larger radii. This curvature is the result of adjacent crystallites within the mineral lamella being tilted by a few degrees to accommodate the bending around the collagen fibril. In addition to curved mineral lamellae, stacks of closely packed uncurved/flat mineral lamellae also exist between adjacent collagen fibrils.

CONCLUSION

Recent advances in analytical technologies reveal that bone is significantly more complicated than previously thought. The unique composition of the extracellular matrix, i.e., the mineralised compartment, affords important mechanical characteristics. Vascular spaces, the osteocyte lacuno-canalicular network, and nanoporosities, i.e., the unmineralised compartment of whole bone, also contribute to the overall function. In the context of bone regeneration, it is no longer sufficient to merely quantify the amount of 'new bone' formed in response to novel biomaterials, rather it must be verified that regenerated tissue resembles native bone in composition, structure, and mechanical competence using correlative multiscale approaches.

DISCLAIMER

None.

CONFLICT OF INTEREST

None to declare.

ETHICAL STATEMENT

Not applicable.

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