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BONE BIOLOGY

Bone mineral as a drug-seeking moiety and a waste dump

A REVIEW

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Bone is a dynamic tissue with a quarter of the trabecular and a fifth of the cortical bone being replaced continuously each year in a complex process that continues throughout an individual's lifetime. Bone has an important role in homeostasis of minerals with non-stoichiometric hydroxyapatite bone mineral forming the inorganic phase of bone. Due to its crystal structure and chemistry, hydroxyapatite (HA) and related apatites have a remarkable ability to bind molecules. This review article describes the accretion of trace elements in bone mineral giving a historical perspective. Implanted HA particles of synthetic origin have proved to be an efficient recruiting moiety for systemically circulating drugs which can locally biomodulate the material and lead to a therapeutic effect. Bone mineral and apatite however also act as a waste dump for trace elements and drugs, which significantly affects the environment and human health.

Cite this article: *Bone Joint Res* 2020;9(10):709–718.

Keywords: Hydroxyapatite, Bone infection, Antibiotics, Bone mineral, Waste dump

Article focus

- This review article provides a historical background on the structure of the bone mineral hydroxyapatite (HA) and its capacity to bind different agents.
- The article explores how different chemicals bind to HA and the concomitant effect on human health and the environment.

Key messages

- Due to the chemical affinity of a broad spectrum of drugs (including antibiotics, bisphosphonates, and radioactive tracers) to HA, it acts as a recruiting platform for systemically administered agents resulting in local targeted drug delivery.
- HA also acts as a waste dump for several agents in nature and the human body, leading to unwanted harmful side-effects.
- Stringent measures should be taken to measure the accretion and safety of contaminants in bone mineral.

Strengths and limitations

- An innovative approach for drug accretion to particulate HA is described.
- This article is not a systematic review but gives a perspective on how hydroxyapatite binds different agents and acts as a recruiting moiety.

Introduction

Apatite is a generic name for a large group of phosphate-containing minerals, essential to providing structural support in living and dead material in nature. Apatite existed before life started and was a prerequisite for the evolution of single cell organisms into vertebrates. Hydroxy(l)apatite (HA – $Ca_5(PO_4)_3(OH)$), often stated as $Ca_{10}(PO_4)_6(OH)_2$ due to its hexagonal crystallographic symmetry, is the stoichiometric form of the inorganic mineral phase in bone and tooth (Figure 1a). Stoichiometric HA is rare in nature; geologically fluorapatite (FAP, $Ca_5(PO_4)_3F$), chlorapatite (CAP, $Ca_5(PO_4)_3Cl$), and multiple substituted apatites are found in rocks and corals.[1,2] More than 300 apatite-type minerals exist, with elements from the entire periodic table replacing Ca, P, and OH in the fundamental apatite crystal structure.[3] In mammalian bone and teeth, further substitutions are found, including both A and

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doi: 10.1302/2046-3758.910.BJR-2020-0097.R1

Bone Joint Res 2020;9(10):709–718.

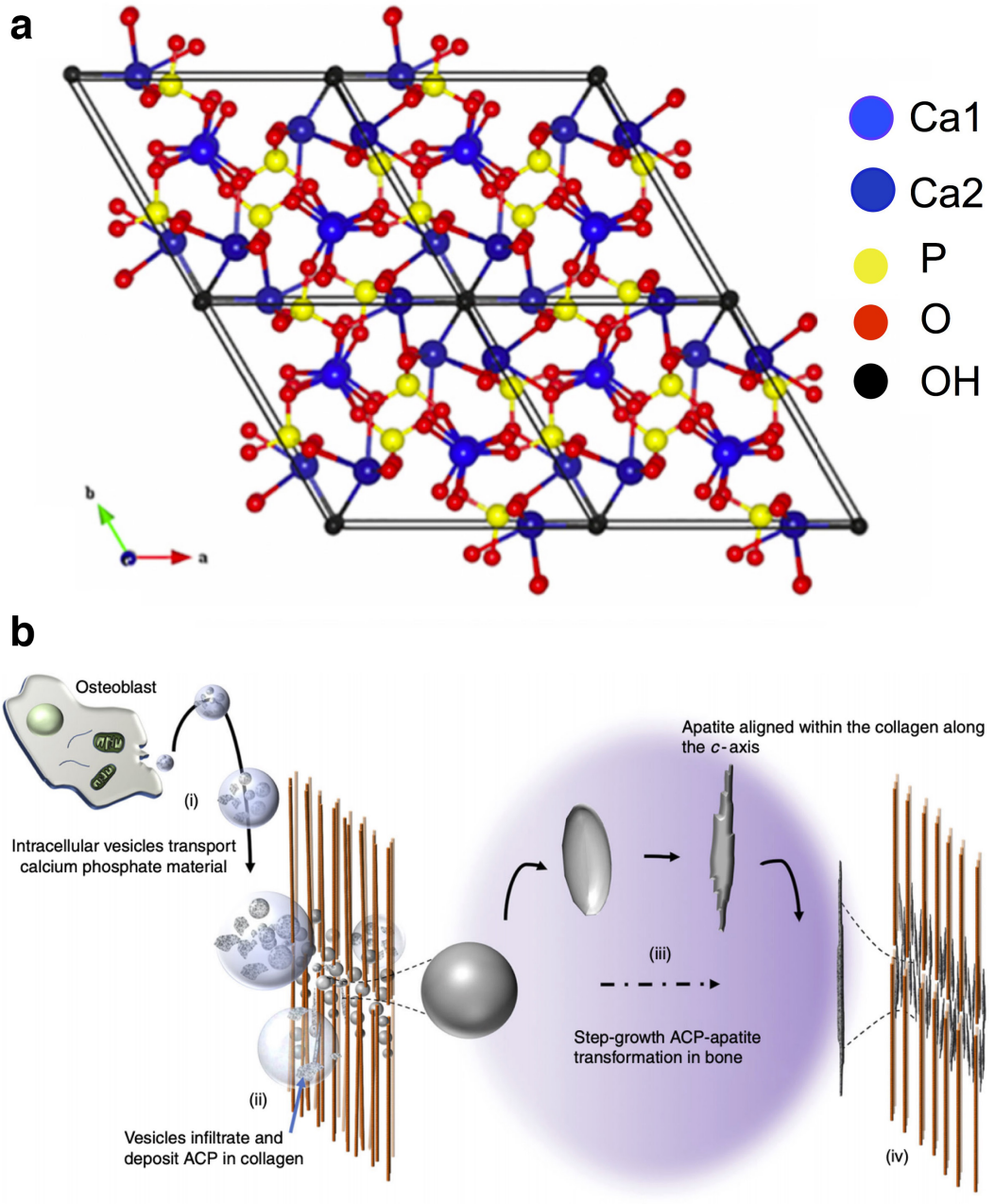

Fig. 1

Hydroxyapatite (HA) structure and speculated mechanism of apatite deposition in bone. a) Unit cell of HA in the (001) plane (reproduced with permission from the ICRP (Fihri A, Len C, Varma RS, Solhy A. Hydroxyapatite: A review of syntheses, structure and applications in heterogeneous catalysis. Coordination Chemistry Reviews. 2017;347:48-76).[6] b) Possible mechanism of HA deposition by osteoblasts in the form of round vesicles (i) containing amorphous calcium phosphate nano-crystals. These vesicles infiltrate the collagen matrix and embed themselves (ii) where they undergo a step-growth (iii) from amorphous calcium phosphate (ACP) to crystalline HA. Finally, (iv) the apatite crystals are elongated and aligned with the long axis of the collagen fibers (reproduced from Lotsari A, Rajasekharan AK, Halvarsson M, Andersson M. Transformation of amorphous calcium phosphate to bone-like apatite. *Nat Commun*. 2018;9(1):4170.[7] Ca, calcium; P, phosphorus; O, oxygen; OH, hydroxyl.

B position carbonate substituted apatites ($Ca_{10}[(PO_4)_{6-y}(CO_3)_y][(OH)_{2-2x}(CO_3)_x]$).[4,5]

Human bone consists of approximately 55% minerals, 35% collagen, and 10% water by weight.[8] The mean annual bone turnover in a healthy adult has been approximated to be about 3.6% with clear differences between cortical and metaphyseal bone as reported by the International Commission on Radiological Protection in 1972.[9] The process is continual and is started by osteoclast progenitors cleaning up old or damaged bone by a series of acidic and enzymatic treatments of old bone/bone mineral followed by infiltration of osteoprogenitor cells differentiating into HA-producing osteoblasts. HA is formed within the collagen fibrils down at the atomic

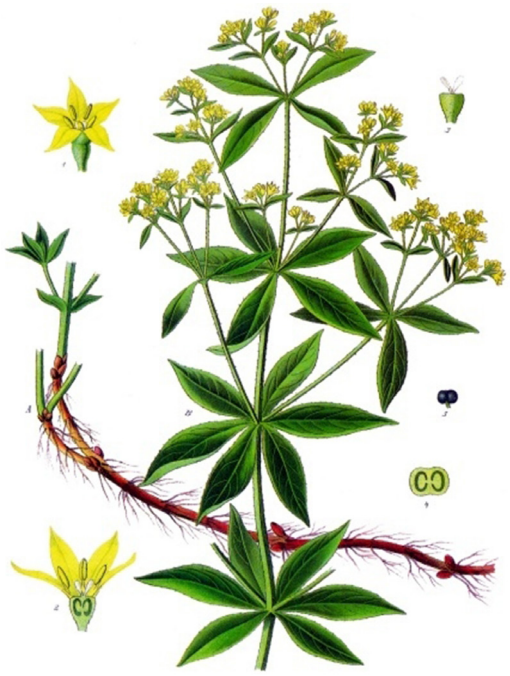

Fig. 2

Rendering of the *Rubia tinctorum* (the rose madder) by Franz Eugen Köhler (Original source: Köhlers Medizinal Pflanzen, 1897).

scale.[7] Cells send out vesicles containing calcium and phosphate ions, deposited within the collagen network. A series of biochemical processes lead to the formation of an ordered, crystalline, but non-stoichiometric HA structure (Figure 1b). The nano-apatite crystals located in the collagen fibrils however make detailed analysis of trace elements in bone mineral difficult. The mineral and collagen content are commonly not extracted directly from the bone, but from ash, which also contains trace elements from the collagen.

The most highly mineralized bone recorded, with a mineral content of 96%, is the rostrum (upper jaw bone) of the whale *Mesoplodon densirostris*, which can be used for understanding the chemistry of bone mineral.[8] Recently an extensive bone mineral content analysis was applied using both bulk analysis (radiograph fluorescence, thermogravimetry, and carbon content) and elemental mapping (electron microprobe). These analyses showed that the mineral in the rostrum on average had a composition of $(Ca_{8.40}Mg_{0.20}Na_{0.54})[(PO_4)_{4.87}(CO_3)_{1.13}](OH)_{0.87}$, different from geological HA $(Ca_{10}(PO_4)_6(OH)_2)$ as the calcium is also replaced by magnesium and sodium while phosphate and hydroxyl groups are substituted by carbonate moieties as well.[10]

Tooth enamel is typically 97% mineral, 1% enamelin, a glycoprotein, and 3% water by weight.[11,12] Again, the mineral phase is rarely stoichiometric with the most common substitution being fluorine-forming fluorapatite, which better resists the development of caries by providing extra protection to the teeth in acidic conditions.[13] Dentine, the hard-inner structure of teeth, like bone, contains typically 40 vol% tooth mineral, which corresponds to 60 wt% mineral.[14]

Hydroxyapatite and its chemical interactions: a historical perspective

Besides being essential in high load-bearing structures like bones and teeth, HA has a remarkable ability to bind molecules, owing to its crystal structure and chemistry. The first report of a substance binding to bone was by Leminus in 1567, describing that the root of the madder plant made bone red (Figure 2).[15]

Almost 200 years later, Belchier in 1736 noticed at an evening dinner that the pork he was served had red bones and was later informed by the host that the pigs had been fed mashed madder root.[15] This was the first report of a systemically administered agent traced in bone. John Hunter, in the late 1700s, studied bone growth by intermittent feeding of pigs with madder root.[16] Hunter observed white and red bands in the growth zones of the pig mandible. More recently, Hoyte in the 1950s used Alizarin (1,2-dihydroxyanthraquinone), an ingredient of the madder root, for vital staining of bone.[17] Based on its interaction with bone, Alizarin is still used today for dynamic histomorphometric studies of mineral apposition.[17,18]

Apart from Alizarin, several other substances have affinity for apatite. In 1935, while working with Niels Bohr (Nobel Prize winner in Physics, 1932) in Copenhagen, Georg de Hevesy, a Hungarian chemist, published the first study on radioactive bone mineral tracers in rats with ^{32}P sodium phosphate.[19] The results showed dynamic bone turnover and continuous uptake of the phosphorus atoms. He is regarded as the founder of radioanalytical chemistry, radiograph fluorescence analysis, father of nuclear medicine, and received the 1942 Nobel Prize in Chemistry. In 1950 in Lund, Sweden, influenced by Niels Bohr and his son Hans Bohr, Göran Bauer, together with Arvid Carlsson, the future 2002 Nobel Prize winner in Medicine, systemically administered a bone mineral-seeking radioactive tracer, ^{45}Ca, to study bone accretion and metabolism.[20,21] Even before the availability of MRI and CT imaging, they and others used bone-seeking radioactive isotopes such as ^{85}Sr for identifying bone turnover. This eventually paved the way for ^{99}Tc scintigraphy, now commonly used for detection of osseous infections, tumours, or other metabolic disorders. Further, with the advancement in chemistry/radiosynthesis techniques and modern imaging equipment, the same tracer ^{99}Tc has been coupled with hydroxy diphosphonate (^{99}Tc-HDP) or methylene diphosphonate (^{99}Tc-MDP) to study mineralization of human stem cells in vitro[22] and bone turnover in vivo in humans.[23]

Hydroxyapatite as a drug-seeking moiety

The most well-known bone-seeking drugs are the bisphosphonates, and their therapeutic use was found by Herbert Fleisch in the 1960s.[24] The bisphosphonates

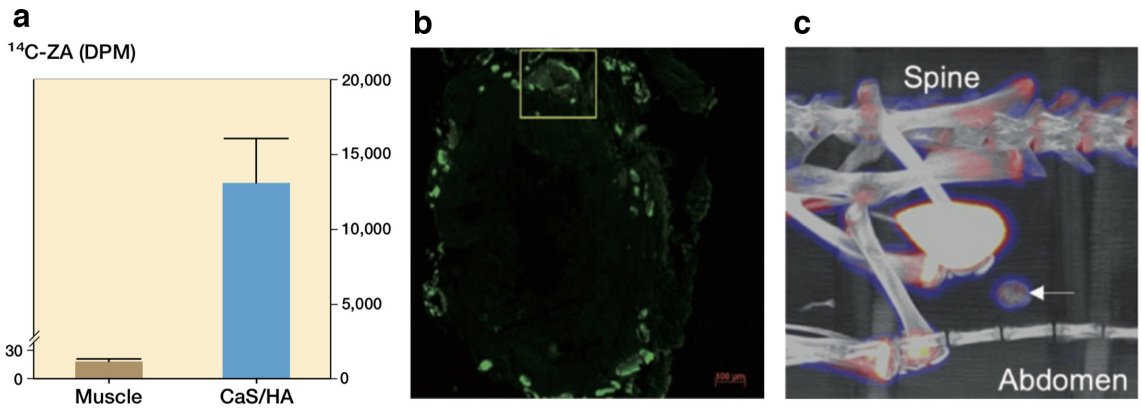

Fig. 3

Hydroxyapatite (HA) as a recruiting platform for systemically circulating drugs in rats. a) Uptake of 14C-zoledronic acid (ZA) in a pellet of calcium sulphate (CaS)/HA implanted in the abdominal muscle pouch. ZA was injected subcutaneously, two weeks post-implantation of CaS/HA biomaterial and animals were euthanized 24 hours later. b) and c) show the uptake of antibiotic tetracycline (injected subcutaneously) and radioactive tracer 18F (intravenous injection) in a pellet of CaS/HA biomaterial placed in the abdominal muscle pouch. Tetracycline uptake was measured using fluorescence microscopy while ^{18}F uptake was accessed using positron emission tomography-CT. Figure has been modified from Raina et al.[28]

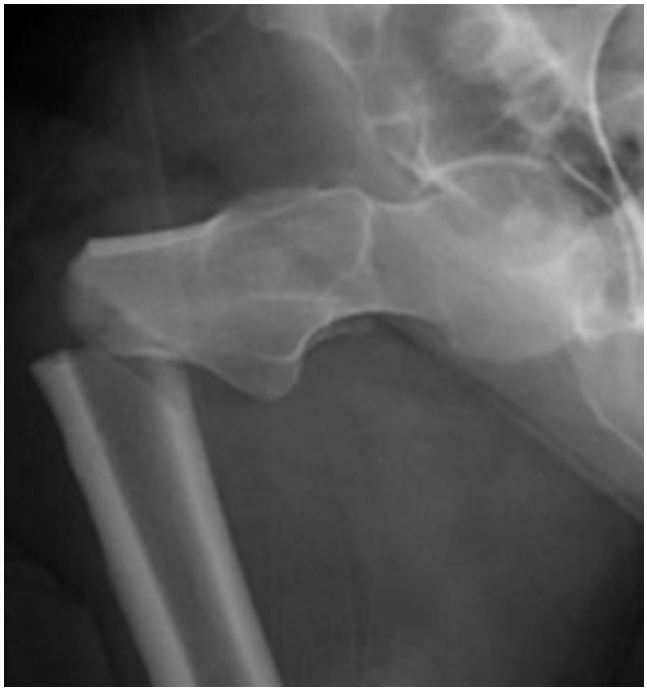

Fig. 4

Anteroposterior radiograph of an atypical femoral fracture caused by the prolonged use of bisphosphonates. Figure is reproduced from Lloyd et al.[32]

are synthetic, non-hydrolyzable analogues of inorganic pyrophosphates having carbon as a bridge between two phosphate groups, resulting in high mineral-binding capacity. Of the antiresorptive bisphosphonates available today, those containing nitrogen in the heterocyclic ring, for example zoledronic acid, are 10,000 times more potent than non-nitrogen-containing bisphosphonates.[25,26] Bisphosphonates are today widely used for retarding osteoporosis by acting on osteoclasts and slowing bone resorption.[25,27]

In our own studies, we have recently implanted stoichiometric synthetic particulate HA in an ectopic muscle pouch model in rats.[28] Two weeks later, the animals were systemically administered zoledronic acid (ZA), which sought the HA particles in the abdominal muscle pouch (Figure 3a).[28] The HA particles could even be reloaded and the local ZA accretion in the implanted HA pellet increased with reloading.

In another long-term in vivo study, radioactive ZA was added to apatite particles implanted in osteoporotic femoral necks in rats. Radioactive active ZA was measured in the synthetic HA particles as well as in the contralateral femur when the animals were euthanized at six months.[29] It appears that bisphosphonates not only bind to the mineral, but remain bound in the bone for an extended period, potentially for years. The drug is eventually released in small amounts by osteoclast activity, only to be recirculated and rebound into vesicles that precipitate into new calcium phosphate mineral.

In several osteoporotic animal studies, we have administered ZA both systemically and locally in multiple anatomical locations, but have never been able to show a positive effect on either bone formation or biomechanical properties of ZA on cortical bone[30] unlike cancellous bone.[31] In patients treated long-term with bisphosphonates, a rare complication is the atypical femoral fracture, a low-energy diaphyseal fracture in a weight-bearing limb (Figure 4).[32] The probable cause is decelerated osteoclastic activity and delayed or even aborted bone remodelling which, without the natural bone repair system, allows a 'critical length' crack to develop in the cortical bone.

Antibiotics in bone mineral and infection

Tetracycline was the first antibiotic that was traced in bone after systemic administration as reported by Andre[33] in 1956. Fluorescence studies have later verified

that bone, enamel, and dentine incorporate tetracycline, leading to discoloured teeth and bone in foetuses, children, and adolescents.[34,35] Perrin[36] confirmed in 1967 that tetracycline strongly binds to HA, including mineral in dead bone, and proposed a chemical interaction between the mineral phase and tetracycline. HA can also absorb tetracycline in solution.[37] We have recently shown that systemically administered tetracycline is capable of seeking and binding to a microparticulate apatite moiety implanted in a muscle pouch (Figure 3b).[28] HA-binding substances can bind the substrate either by sequestering the calcium/phosphate ions or by forming hydrogen bonds.[38] Hydrogen bonding can be efficient if the binding agent contains hydrophobic moieties. The affinity of an antibiotic to apatite particles would foremost depend on its chemical structure, i.e. binding capacity to the calcium, phosphate, and hydroxyl groups of HA, and the number and size of the particles.

During the last 50 years, aseptic and antiseptic measures have focused on prevention and hindering bacteria from colonizing and forming biofilms on implanted devices.[39] If a deep bone or joint infection (DBJI) occurs, the implant is commonly removed and temporarily exchanged with an acrylate spacer containing very high levels of antibiotics such as gentamycin and vancomycin. Frequently, bacteria can still be found in the surrounding soft tissue and bone when the spacer is removed during a second stage, at prosthetic reimplantation.[40] The costs associated with reoperations in the case of a revision total hip arthroplasty (THA) can be as high as £50,000 GBP, as recently reported by Ahmed et al[41] from the UK.

A novel treatment in DBJI is to implant a local microparticulate biphasic calcium sulphate-apatite carrier containing antibiotics, i.e. gentamycin or vancomycin, released in a targeted location in supraphysiological concentrations 100 to 1,000 times above the minimal inhibitory concentration (MIC) levels, but avoiding systemic side effects.[42,43] The carrier is injectable and sets in situ in the defect created by radical surgical debridement. A success rate of up to 95% healing has been reported without infection recurrence in chronic longstanding osteomyelitis.[44] Gentamycin and vancomycin, in contrast to tetracycline, do not bind to HA, and consequently are completely released within a month, following the resorption of the soluble calcium sulphate (CaS) phase in the CaS/HA carrier.[42,43] Even without the use of a carrier, prolonged use of antibiotics such as gentamycin or vancomycin have been reported to have no impact on bone turnover markers such as carboxy-terminal collagen crosslinks (CTX) or osteocalcin in humans.[45] We have not been able to find any studies reporting a negative effect of long-term antibiotic usage or accumulation in bone with the exception of tetracycline. Recurrence of infection is reduced both in chronic osteomyelitis as well as in PJI, both with non-HA-binding antibiotics such as penicillin[46] or with HA-binding antibiotics such as rifampicin,[47] even with prolonged treatment.

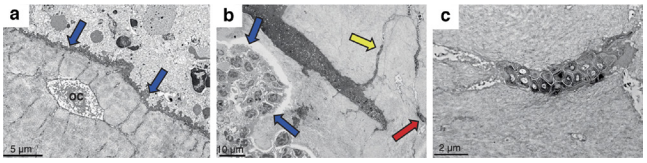

Fig. 5

Bacterial attack on bone via the osteocyte lacunocanalicular network. a) and b) show transmission electron microscopy (TEM) images of the cortical bone from a mouse model of implant infection created using *Staphyloccocus aureus*. *S. aureus* develops an attack front to eradicate the bone lining cells (blue arrows) and embedding the osteocyte (OC). After compromising the cortical bone, the bacteria then colonize the osteocyte lacunocanalicular network (yellow arrow) and migrate within the canaliculi by expanding the structure to reach other osteocytes (red arrow in Fig. 5b). They simultaneously demineralize the bone matrix. c) An osteocyte lacuna inhabited by infiltrating *S. aureus* bacteria. Marker bars are: a) 5 µm (6,000× magnification); b) 10 µm (1,800× magnification); and c) 2 µm (12,000× magnification). Image is reproduced from Masters et al.[53]

So far, no one has provided a full rationale as to why certain systemic antibiotic combinations have superior effect when it comes to biofilm-associated bone infections; we suggest that the reason could be the difference in antibiotic accretion in bone mineral.[48] As a possible coincidence, the recommended and second level systemic antibiotics in DBJI, such as rifampicin and daptomycin, all have chemical structures that allow them to bind to apatite.

Qayoom et al[49] recently reported in an in vitro study that rifampicin used for DBJI and tuberculosis, added to a biphasic calcium sulphate/HA carrier, binds to the HA phase. They found that 50% of the antibiotic remained in the material at three months, contrary to the non-binding anti-tuberculosis antibiotic isoniazid, which was released within the first month. This may explain the clinical rationale behind the proven efficacy of systemic antibiotic treatment in DBJI, using a combination of vancomycin/gentamycin with rifampicin.[50]

It is well known that mycobacteria in bone tuberculosis can be dormant in their 'bone sarcophagus' for decades and then relapse. The senior author (LL) has in his clinical practice seen a tibial diaphyseal osteomyelitis recurring at the original location with *Staphylococcus aureus* after 50 years of dormancy. In the literature, an osteomyelitis case was published with a genetically identical *S. aureus* strain recurring after 75 years of quiescence.[51] The bone marrow, surrounding soft tissue, and the glycocalyx on the surface of implanted hardware are all well-known reservoirs for bacteria. Bacteria can also be internalized in bone cells such as osteoblasts or osteoclasts but probably only during the acute or subacute phases of an infection and generally do not persist.[52] However, in long-standing chronic osteomyelitis, bacteria have recently been shown to reside sessile in the osteocyte-lacuna canalicular network (OLCN) in the cortical bone (Figure 5).[53] This is a plausible long term 'hide away' and could explain why bacteria could survive in bone lifelong. This cortical bone OLCN sarcophagus needs to be opened and 'the sleeping

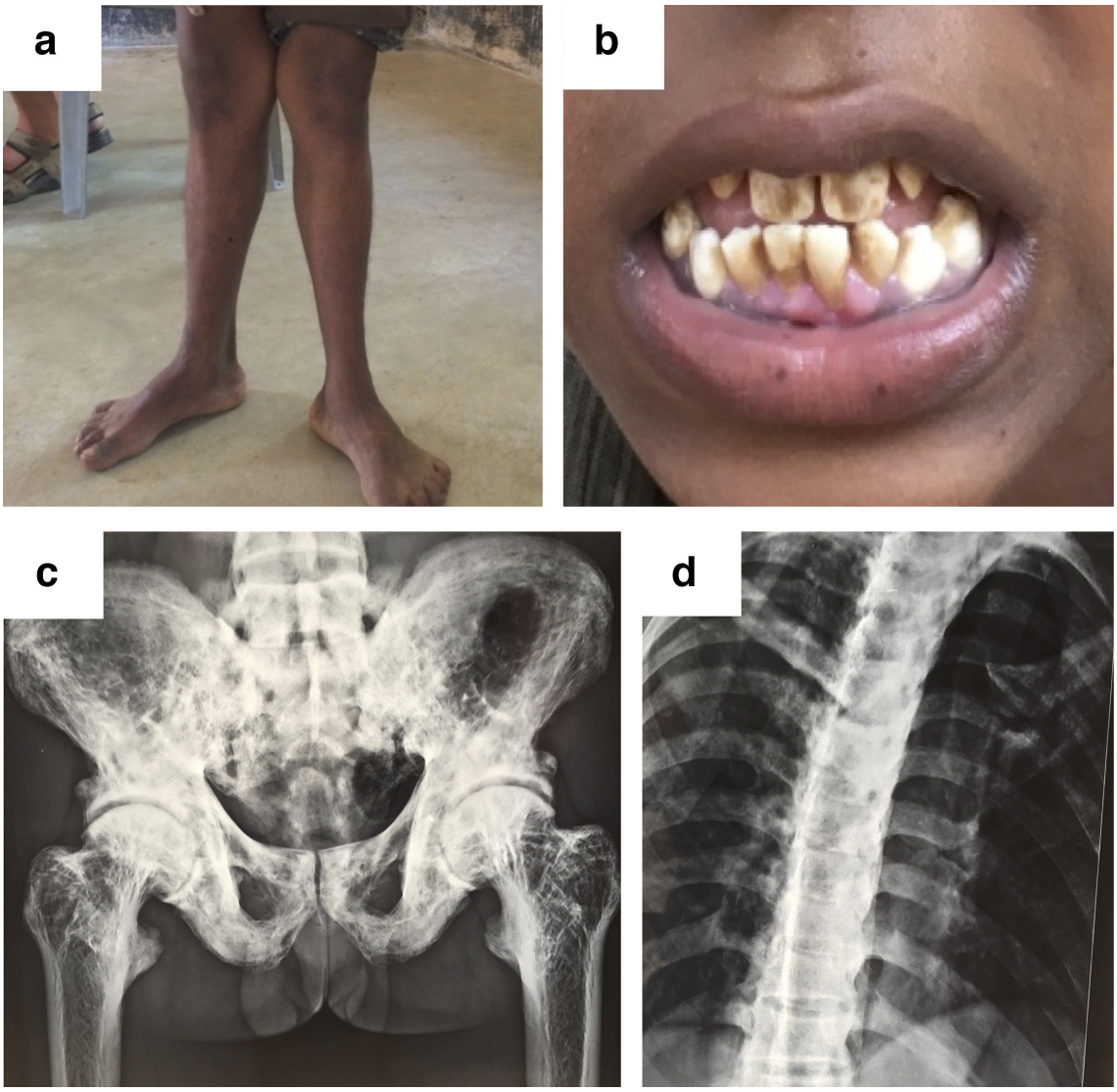

Fig. 6

Anteroposterior radiographs showing the skeletal effects of fluoride poisoning through drinking water in humans. a) Knee deformity in a 12-year-old boy and b) fluoride-induced discolouration of teeth. c) and d) show radiographs of patients exposed to high fluoride levels showing dense sclerotic pelvic bone and spine. Unpublished images used with permission from Prof. Urban Rydholm, Lund University Hospital, Lund, Sweden.

beauty' i.e. dormant sessile bacteria attacked using new treatment methods.

A possible future development could be to use a local HA or other calcium phosphate carriers preloaded or mixed with an HA-binding antibiotic, and combine it with a non-binding antibiotic added to a material locally. An initial high, effective, and sustained local release of, for instance, gentamycin or vancomycin will be achieved.[42,43] By osteoclast resorption, a HA-bound antibiotic, such as rifampicin or a tetracycline, will be continuously released in the OLCN and thereby poison the sessile bacteria in the cortical bone sarcophagus in the long term. Even more intriguing is the recent finding that a local moiety containing tens of millions of HA particles can be loaded and reloaded after implantation by systemic drug administration.[28,54]

Bone: a waste dump that affects health

Fluorosis an environmental bone dump disease. Fluorine binds rapidly to carbonated HA in bone and is found in small amounts in nature, in ground water leaking out from fluorapatite (FAP) in rocks. HA precursors are octacalcium phosphate (OCP) and in the case of long-term high F^- exposure, the F^- ion will be accumulated into bone and teeth by the formation of fluorapatite (FAP) ($Ca_5(PO_4)_3F$) instead of HA.[55,56]

Fluorine can in certain endemic areas reach toxic levels in drinking water resulting in severe growth deformities in children (Figure 6a and 6b). In adults, fluorine causes 'marble bone disease' with severe secondary osteoarthritis (Figure 6c and 6d).[57,58] The consumption of drinking water with a fluoride concentration higher than

the World Health Organization's (WHO) recommended limit of 1.5 mg/l[59] is a health concern for more than 250 million people today worldwide and an endemic causing serious disability in, for example, India.[60] In many industrial countries, although controversial,[61] fluorine is added to drinking water. It is also an ingredient in toothpaste to reduce dental caries and recommended by dentists as mouthwash, especially for children. FAP is more resistant to the low pH produced by dental caries than HA.

Triclosan and apatite: an environmental and health concern? Triclosan (TC) is a phenyl ether with a broad antibacterial spectrum widely used in soap, surgical sutures, toothpaste, deodorants, clothes, sun protectors, etc. The impact on health is the subject of debate - over-the-counter soap containing TC has been banned in the USA and Canada - as is the antibacterial effect of TC. There is no conclusive evidence that sutures containing TC have any effect on deep surgical site infection (SSI), and very limited evidence for preventing superficial infection. In 2019 after a large randomized controlled trial, TC was not found to prevent superficial SSI and it was concluded that the use of TC-coated sutures in hip and knee arthroplasty does not lead to a reduction in the rate of SSI.[62]

In several studies, TC has been found in water,[63] and in animal and human tissue.[64] HA has been shown to interact with TC via hydrogen bonding and electrostatic interactions. Previously, Gilbert and Watson[65] showed that TC binds to saliva-coated enamel. Coral reefs, like bone, are made up of calcium derivates, mainly calcium carbonate, and it has been reported that sunscreen ingredients, including TC, accelerate coral bleaching (Figure 7).[66] In 2020 Adamson and Shinkai[67] reported that substances in common sun lotions are being absorbed through human skin. The reported blood levels exceeded Food and Drug Administration (FDA) safety levels for several days.[67,68] It was concluded that this needs to be investigated further to exclude any harmful effects. Countries with tourist beaches and coral reefs are therefore increasingly banning TC-containing sunscreens, to save the coral reefs.

In a large recent study, women with high TC levels in their urine, compared with those with low levels, suffered from osteoporosis as indicated by a low bone mineral density (BMD) at several anatomical locations.[69] The study does not prove a causal relation between TC in urine and osteoporosis, or the risk of sustaining a fragility fracture, and long-term prospective studies with multiple timepoints are needed, but an explanation could be that women with lower BMD may be in a catabolic phase due to increased osteoclast resorption releasing TC bound to apatite. TC chemically mimics oestrogen, thus affecting bone turnover. TC binding to HA needs to be explored both in vitro as well as in vivo, and degradation and release measured. The level of TC in animal, human apatite, and coral apatite should be studied in exposed areas and compared to areas with non-contaminated water. In addition, the regulation of TC should be tightened, at least in consumer goods.

Fig. 7

Image of a bleached coral reef in the Great Barrier Reef, courtesy of Jayne Jenkins, reproduced with permission.

Husbandry contaminating the environment and human bone mineral with antibiotics. Husbandry accounts for three-quarters of all antibiotics consumed. The rationale is mainly economical and aims to increase the weight and improve the lives of cattle, pigs, and poultry.[70] In recent years, farmed fish have been given antibiotics to reduce the infection burden during their short life cycle. Resistant bacteria, crossing over between husbandry and humans, have been documented: a recent analysis revealed that from 2000 to 2018, the proportion of antimicrobials showing more than 50% resistance increased from 0.15 to 0.41 in chickens, from 0.13 to 0.34 in pigs, and plateaued between 0.12 and 0.23 in cattle.[71] A global map based on data for the antibiotic use and development of resistance in husbandry is available and shows middle-income countries to be the worst affected (Figure 8).[71] Resistant bacteria travel light and can cross borders without passports. Regulation has improved and in the USA, use of tetracycline, a common antibiotic used in cattle that is given at any time during the animal's life cycle, has been reduced from 80% to 40%. In Germany, probably the most regulated country in Europe, farming administers antibiotics seven times more often

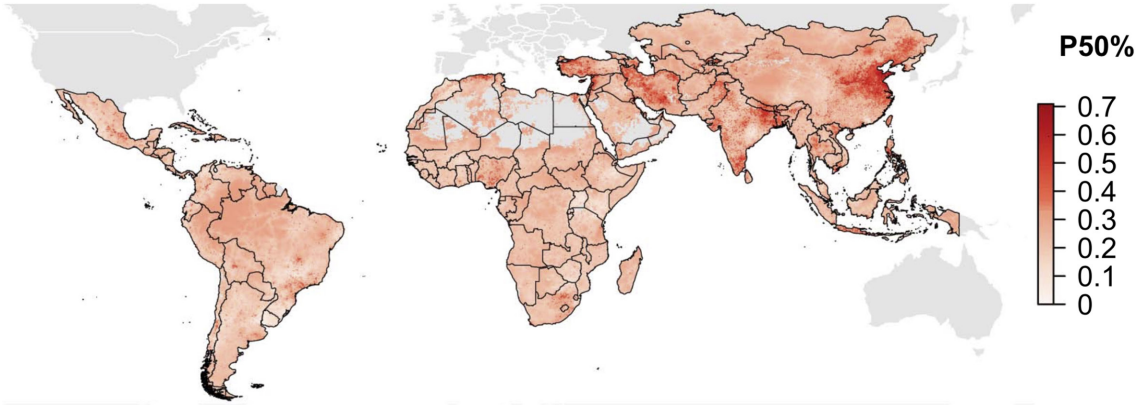

Fig. 8

World map on antibiotic resistance from van Boeckel et al[71]. This map emphasizes P50, the proportion of bactericidal antibiotics which have a resistance above 50%. Only low- and middle-income countries are reported here. Image is reproduced from van Boeckel et al with permission.[71]

than in Sweden, although still on a low level. In some countries tetracyclines are still routinely added to daily feeds. Tigecycline, a commonly used tetracycline, is also used in humans. The concern is that tetracyclines bind to HA and remain in the bone for years.[72] Thus, even if no antibiotic is given several weeks before slaughtering, the bone mineral remains loaded with antibiotics, which is highly relevant if the bone is industrially processed.

Bone settlers as waste dumpers. There is a large industrial sector with production based on the mineral and collagen components of husbandry bone post mortem.[73] Collagen is extracted and used in several industrial processes, including food production, as gelatine. For bone, heating to 200°C and/or acid softening is common, followed by grinding into a bone powder. There are large differences in thermostability between antibiotics used in husbandry: for instance, doxycycline, another tetracycline, remains stable even above 150°C.[74] The bone meal is a valuable additive used in pet food and as fertilizer for vegetables.[73] In some countries it is added as a meat expander/substitute in hamburgers.

There is a lack of studies on bone meal as a source of secondary antibiotic contamination, and a lack of data regarding degradation, release, and resistance pattern in humans, the soil, and water. There is also a dearth of research on trace elements bound to HA in husbandry bone both in living animals and post mortem in the meat industry. We suggest that control of antibiotic food contamination extends to hard tissue, as well as the regulation of the 'bone settlers' production technology.

Antibiotic water contamination - a harmful circle. The rise in antibiotic-resistant bacteria is a global health emergency that may kill ten million people by 2050, according to a UN report.[75] In Europe 33,000,[76] and globally 700,000,[75] people were recently reported to die annually due to an antibiotic-resistant bacterial infection. Researchers recently tested 711 rivers and lakes in 72 countries and found antibiotics in 65% of them.[77,78] In 111 of the sites, the concentrations of antibiotics exceeded safe levels, possibly contributing to the development of bacterial resistance. Lower-income countries generally had higher antibiotic concentrations in rivers and were often lacking the technology to remove the drugs.

Samples taken from rivers in Europe, such as the Thames in the UK and the Danube in Austria, also showed severe antibiotic contamination for seven antibiotics at nearly four-times the 'safe' level. At worst, it was 300 times over the safe limit in some rivers.[78] Some concentrations are even so high that drinking three glasses daily from the Danube could potentially be sufficient as oral antibiotic treatment for an upper respiratory infection. Some of the antibiotics, such as tetracyclines, may again result in animals drinking the water with their bone reactive mineral phase acting as a waste dump. An antibiotic-induced minor change in the microbiome may increase the risk for an infection and postoperative DBJI.[79]

In conclusion, we have described novel binding mechanisms for drug accretion to micro- and nano-apatite particles biologically activating the material. By systemic administration, pharmaceutical agents can seek and biomodulate a local moiety with apatite particles acting as a Trojan rechargeable drug reservoir. The chemical interactions between specific drugs and apatite, including non-stoichiometric HA found in bone, are however also responsible for a broad range of potential harmful effects. Antibiotics administered in husbandry pose considerable crossover risks of accrued drugs from animal bone to humans affecting the microbiome.

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Funding statement:
- No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article

ICMJE COI statement
- L. Lidgren reports board membership on Bone Support AB, Sweden and Orthocell Australia. L. Lidgren and M. Tägil report an institutional grant from the Swedish Research Council, unrelated to this study.