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Fluoride and Mineralized Tissues

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ABSTRACT: This review focuses on the interaction of fluoride with the material properties of bone and teeth, which is of clinical, scientific, and public health interest. These tissues are composed primarily of collagen (protein) and hydroxyapatite (mineral), and their mechanical function depends on the properties of the constituents, their proportions, the interface, and the three-dimensional structure. Changing any of these may have clinical consequences. Fluoride interacts with mineralized tissues in a number of ways. At low doses, the fluoride may be passively incorporated into the mineral, stabilizing it against dissolution; this is one of the mechanisms by which municipally fluoridated water reduces the incidence of dental caries. At higher doses, such as those used for treatment of osteoporosis, the fluoride may alter the amount and structure of tissue present, including altering the interface between the collagen and mineral. At very high doses, skeletal and dental fluorosis occurs, characterized by debilitating changes in the skeleton and by marked mottling and discoloration of teeth, which may be accompanied by increased wear of the enamel. These effects have been observed in communities where the local drinking water has naturally high fluoride levels. Understanding the influence of fluoride on mineralized tissues is, therefore, of considerable significance.

KEYWORDS: fluoride, bone, teeth, dentin, enamel, fluorosis, mineral, mechanical, properties, water fluoridation, public health

I. INTRODUCTION

Fluoride is an element that occurs naturally in the human diet and is found in many water sources as well as a range of foods. The interaction of fluoride and mineralized tissues in the body was first recognized early in the 20th century as a result of toxic fluoride exposure with the dual observations of endemic dental fluorosis in regions where the water supplies were naturally fluoridated at high (> 4 ppm) levels[1] and of increased radiodensity of the skeleton in individuals exposed to fluoride.[2] Since then, the effects of fluoride on bones and teeth have been extensively investigated.

The observation that increased fluoride exposure decreased the risk of dental caries led to the widespread fluoridation of municipal water supplies, beginning in 1945.[3] Water fluoridation is widely considered to be one of the most successful public health disease prevention pro-

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grams ever initiated[4] and has been endorsed by more than 150 science and health organizations, including the World Health Organization.[5] Investigation of the effects of fluoride on teeth has, therefore, focused on finding appropriate levels of fluoride exposure to minimize the incidence of dental caries and on understanding the causes and effects of dental fluorosis due to overexposure. This paper will discuss the effect of fluoride on the physical properties of teeth over a range of doses centered around those used in municipal water fluoride.

The relationship between fluoride and bone is much more complex and well-investigated. Like many other biological materials, including the dentin and enamel that form teeth, bone has a complex, hierarchical structure. The tissue is not static; it is in a constant state of remodeling, as existing bone is removed and replaced with newly formed bone by a set of specialized cells. Fluoride exposure can, therefore, affect the composition of bone, its three-dimensional structure, and the bone cells; needless to say, these changes can have profound implications for the mechanical properties of these tissues. Investigation on the effects of fluoride and bone has, therefore, included low doses (consistent with municipal water fluoridation), a middle range of therapeutic doses that were administered as a therapy for osteoporosis, and high doses that resulted in skeletal fluorosis.

After a brief background on sources of fluoride in the human diet and the fluoride metabolism in the body, this paper will focus on how fluoride affects the material (structural and mechanical) properties of bone and of teeth. These effects occur at levels ranging from the molecular structure to the mechanical properties, and some are of considerable public health importance.

II. AN OVERVIEW OF FLUORIDE METABOLISM

Fluoride is ingested through fluoridated water, food, and oral care products, such as toothpaste and fluoridated mouthwash. Municipally fluoridated water in North America generally has a fluoride concentration of approximately 1 ppm (1 mg/L), and the average adult consumption of water is 1.5 L per day. Many foods contain fluoride, notably fish and other marine products, and tea.[6] Fluoride ingestion from food in individuals over the age of 12 years has remained constant at approximately 0.4 mg/day.[7] Increasingly, however, the diet of individuals living in nonfluoridated areas comprises beverages and foods prepared with fluoridated water.[8] Individuals, especially children,[9] also ingest fluoride from oral care products, particularly toothpaste. On average, 1 g of

toothpaste is used per brushing, and adults typically ingest about 25% of it.[10] In North America, toothpaste is fluoridated at 1000 to 1100 ppm, resulting in ingestion of 0.25 to 0.75 mg/day of fluoride. As we discuss below, ingested fluoride can have systemic effects on mineralized tissues as they form, so children are more vulnerable to fluoride overexposure (the American Dental Association recommends that children only brush with toothpaste under supervision, and special low-fluoride toothpastes for children are also available). Finally, fluoride supplements are often provided to residents of nonfluoridated areas.[11] Overall fluoride consumption, therefore, includes a range of other sources in addition to fluoridated water, with the estimated adult total intake of fluoride ranging from 1.2 to 2.2 mg/day.[10]

Ingested fluoride is absorbed rapidly and almost completely in the body. While some absorption occurs in the mouth (for example, of topical fluoride preparations), the majority of fluoride absorption occurs by diffusion, primarily in the stomach and the proximal small intestine.[12] The absorption is very rapid, with a half-life of approximately 30 minutes, so peak plasma concentrations are reached within an hour.[13] Approximately 80% to 90% of ingested fluoride is absorbed, and the remainder is generally excreted in the feces.[14]

Once absorbed, fluoride enters the plasma. It does not bind to proteins or any other constituent.[15] The fluoride distributes between two compartments: the blood and soft tissues from which it is cleared within a few hours, and the calcified tissues in which fluoride can be sequestered with a half-life of years.[14] In the soft tissues, the concentration is proportional to the plasma concentration.[16] Calcified tissues clear fluoride from the plasma at an extremely high rate; in adults, approximately half of ingested fluoride becomes associated with the bones within 24 hours (the remainder is excreted). A higher proportion of fluoride is retained in the calcified tissues of young children due to their high bone modeling rates.[10] Fluoride incorporation and its relationship to bone remodeling are discussed in detail below.

Fluoride is generally not considered to be under homeostatic control in the body, and therefore, the plasma concentration is a function of the ingested fluoride. In healthy adults for whom fluoridated drinking water provides the major source of fluoride, the fasting plasma concentration of fluoride (in μmol/L) is numerically equivalent to the concentration of the fluoride in the water (in ppm), clearly indicating that the plasma concentration of fluoride is not regulated in the body.[10]

Fluoride that is not taken up by the skeleton is excreted into the urine by the kidneys. Its renal clearance rate is far more rapid than

other halogens; in healthy adults, the clearance rate is approximately 35 mL/min, although the range can vary considerably.[10] The renal clearance of fluoride in young children is much lower than that of adults, ranging from approximately 4 to 9 mL/min largely due to much greater uptake into the forming skeleton [17]. In addition, there is evidence that the uptake of fluoride into the skeleton may be dose-dependent.[18] The reduced renal clearance rate and higher proportional dose contributes to the higher susceptibility of children to adverse effects of fluoride exposure.

The fluoride that is taken up into the calcified tissues is not irreversibly bound; rather, it forms a sequestered pool.[13] Evidence from animal studies suggests that a small amount of fluoride (10%) bound to calcified tissues can be remobilized rapidly, possibly because it is adsorbed on the surface.[19] However, fluoride is generally returned to the circulation much more slowly, as a result of remodeling of the calcified tissue;[13] for example, the half-life of fluoride in bone has been estimated at 8 to 9 years.[20] Accordingly, the clearance of fluoride is closely dependent on the rate of remodeling, which can change with age and disease state, particularly in bone.[21] Finally, the observation that fluoride concentration of bone increases with age[22] suggests that some of the fluoride is released while remodeling is re-incorporated into the bone.[23]

A number of factors can affect fluoride metabolism. Fluoride absorption in the stomach is affected by the presence of elements that form insoluble compounds with fluoride, notably calcium.[10] A high-calcium diet is, therefore, associated with increased fecal excretion of fluoride.[10,24] Calcium-containing liquids are also administered to individuals who have received a toxic dose of fluoride (for example, in industrial accidents). Fluoride absorption also increases with decreasing gastric pH.[12] Finally, the profile of fluoride concentration in the plasma (and the exposure of the calcified tissues to fluoride) is a function of how rapidly it is cleared from the system and on renal function.[12,25] Individuals with reduced renal function (kidney disease) are, therefore, at greater risk for skeletal fluorosis.[26] A number of other factors can affect fluoride metabolism, including altitude, acid-base disturbances, and exercise.[12] Finally, there is evidence of a genetic component to susceptibility to fluoride.[27,28] This wide range of factors that can affect fluoride absorption means the same dose of fluoride may have widely differing effects on the mineralized tissues of different individuals, as discussed further below.

In addition to ingestion of fluoride through normal dietary sources, some individuals may receive higher doses of fluoride for therapeutic reasons. This includes oral fluoride supplements given to residents of

regions with nonfluoridated water sources as a means of reducing dental caries. However, because fluoride is known to increase bone mass, it has been under investigation as a therapy for osteoporosis for several decades.[29] Because of the dose-dependent response of both teeth and bone to fluoride, as well as the wide range of susceptibility to fluoride administration discussed previously, care must be taken to find the appropriate dose level to maximize the positive effects (reduced dental caries, increased bone mass and strength) and to minimize the risk of dental or skeletal fluorosis.

III. FLUORIDE AND BONE

III.A. Bone Biology

1. The Composition and Structure of Bone

From an engineering perspective, bone is a composite material with a complex, three-dimensional architecture. The material of bone consists of an organic matrix reinforced with mineral in approximately equal volume fractions (approximately a 1:3 ratio by weight; the discrepancy stems from the significantly higher density of the mineral). Over 90% of the organic component is type I collagen; the remainder is primarily noncollagenous proteins and small molecules. The mineral component consists of poorly crystalline hydroxyapatite, with a stoichiometric formula of $Ca_{10}(PO_4)_6(OH)_2$. However, the mineral component is not generally found in this stoichiometric form, as a number of ions substitute for the phosphate and hydroxyl groups, including sodium, carbonate, and fluoride. The mineral is in the form of needle-like crystals, 3 to 6 nm in diameter and 20 to 40 nm long. In the composite structure, these crystals lie parallel to the collagen fibres.[30] While the exact nature of the relationship remains poorly understood, it is likely that the collagen fibers epitaxially nucleate mineral growth, resulting in a close apposition between the two components and a strong interface, thereby forming an effective composite with high strength and unparalleled toughness.

Bone tissue that is primarily loaded in compression, such as the spine and the epiphyses (the ends of the long bones), has a spongelike appearance and is termed *cancellous* or *trabecular* bone. This bone has a porous structure, characterized by a complex three-dimensional latticework of plates and struts (trabeculae). The architecture of the structure, including the amount present, the orientation, and the degree of connectivity, contributes to the mechanical strength of the tissue as a

whole. Bone tissue that is primarily loaded in bending, such as the shafts of the long bones, is much denser and is known as *cortical* bone. This bone is characterized by Haversian (longitudinal) canals, which provide passage for blood vessels, enabling the bone to remain vascularized. These canals also provide a locus for remodeling in the cortical bone, allowing the bone tissue to be removed and replaced with new bone in a controlled way. Together, the canals and the surrounding bone material compose the Haversian systems; cortical bone is, therefore, sometimes known as Haversian bone.[30]

2. Remodeling in Bone

Bone is not a static material. Throughout the lifetime of the individual, even after growth is complete, bone tissue is *remodeled*; that is, material is removed and replaced. The biological activity in bone is mediated by two types of cells. Bone is resorbed by osteoclasts, multinucleated giant cells derived from monocytes. They act by first dissolving the bone mineral by creating a highly acidic microenvironment between the cell body and the underlying bone tissue and then digesting the collagen matrix. New bone is then formed by osteoblasts, derived from fibroblast-like mesenchymal precursors, which lay down a collagenous matrix, which is then mineralized. Resorption and formation of bone are normally tightly coupled, with new bone being laid down immediately after resorption is completed. In cancellous bone, this process takes place on the surface of the trabeculae. In long bones, this process occurs longitudinally throughout the bone, along the Haversian canals, resulting in a lamellar appearance resembling tree rings.[31]

As closely coupled as resorption and formation are, they are rarely exactly balanced, and therefore, the amount of bone that forms the skeleton may be increasing or decreasing. During growth, the rate of formation exceeds the rate of resorption considerably. However, after the age of approximately 30 years, the balance shifts slightly towards increased resorption. This results in a gradual decline of bone mass over the remaining lifetime of the individual.[31]

Remodeling exists for several reasons. The first is homeostatic; the mineral in bone serves as a reservoir for calcium and phosphate. More than 99% of the calcium and 85% of the phosphorus in the body is sequestered in the bone. Remodeling provides a mechanism to remove and replace these physiologically important ions as needed. In addition, remodeling aids in maintaining the mechanical integrity of bone. Constant turnover of bone (on average, all the bone in the skeleton is

replaced every 10 years) ensures that fatigue damage does not have a chance to accumulate to dangerous levels. Additionally, there is increasing evidence that this may not simply be an unguided, prophylactic process; bone remodeling has been found to increase in areas of severe fatigue damage.[32] In this case, apoptosis of cells in the damaged areas may trigger the resorption/formation cycle. The remodeling process also provides a mechanism by which the shape or the architecture of the bone can respond to changing loading patterns, whether increased loading or disuse. The hypothesis that the structure of bone directly reflects the applied load is termed *Wolff's Law*.[31,33] In addition, the rate of remodeling is affected by other factors, notably hormones. Sex hormones, such as testosterone and estrogen, are responsible for the growth spurt at puberty as well as influencing bone mass throughout life. At menopause, for example, the cessation of estrogen production leads to more rapid turnover of bone, resulting in accelerated bone loss.

As discussed earlier, systemic fluoride is sequestered rapidly into the bone during both childhood and adulthood; this is due to the ongoing process of remodeling. However, the impact of fluoride on the bone tissue varies sensitively with the dose.

3. Incorporation of Fluoride into Bone

As fluoride is not incorporated into fully mineralized bone and accumulates only in bone formed during the period of exposure, the process of remodeling provides a mechanism for the incorporation of fluoride into adult bone.[34] A mathematical model of fluoride incorporation in the skeleton[35] indicates that the total bone fluoride content is linearly related to the daily ingestion of fluoride in adults up to age 55 years, after which it appears to plateau. However, other research suggests that fluoride can continue to be incorporated into bone through to the 8th decade of life.[22,36,37] As discussed earlier, the incorporation of fluoride can be affected by other factors, notably osteoporosis or impaired renal function. In osteoporosis, resorption of bone exceeds formation, resulting in gradual bone loss, and fluoride is minimally incorporated into bone; in fact, if daily intake is low, patients may actually lose fluoride from bone.[12] Note, however, that it takes approximately 4 times longer to remove the fluoride than to incorporate it, due to the reutilization of the fluoride.[38] Conversely, increased uptake of fluoride by bone results if renal function is impaired because ingested fluoride is not cleared rapidly from the system.[39] Therefore, fluoride levels in bone can vary greatly.[22,36,37] The concentration of fluoride in bone is generally higher

in sites with a higher turnover rate, such as cancellous compared to cortical bone[36,37] or vertebrae compared to the iliac crest.[35]

III.B. Fluoride and the Materials Science of Bone

1. Overview

There are three primary cases in which we are concerned about the effect of fluoride on bone, which correspond to low, medium, or high doses of fluoride. Of considerable public health interest is the effect, if any, of exposure to municipal water fluoridation on the properties of bone; the concern is that the low doses of ingested fluoride, which reduce the incidence of caries, may accumulate in bones and lead to impaired physical properties, expressed as a higher risk of bone fracture. Fortunately, both epidemiological and experimental studies suggest this is not the case, as discussed further below. Larger doses of fluoride are under investigation as a therapy for osteoporosis. Finally, high doses of fluoride result in skeletal fluorosis, which is characterized by pathologically increased bone mass, as well as ectopic calcification of ligaments and joint capsules, resulting in pain or immobilization of joints. While the effects of fluorosis are clearly systemic, in the cases of low or medium exposure, we are primarily concerned with how the fluoride administration affects the mechanical properties of the bone. While bone plays many roles in the body, its mechanical function is certainly the most conspicuous and one of the most important. This is highlighted in patients with osteoporosis, which is characterized not by bone loss per se but by the loss of the mechanical integrity of bone, as manifested by fracture upon minimal loading. Note, however, that we rarely measure the mechanical properties of bone directly (except in experimental work); the clinically significant parameter is *fracture risk*, a measure of how likely it is that a patient will sustain a fracture, or the *fracture incidence* in a population.

The mechanical function of bone depends not only on the amount of bone present but also its organization at the macroscopic (such as the shape of long bones), the microstructural (the architecture of trabeculae or of Haversian systems), and the ultrastructural (the intimate association of collagen and mineral) level. As well, the properties of bone tissue depend on the degree of and the distribution of mineralization in the material (that is, the relative proportions of the mineral and the collagenous component). One concept that is commonly used in the field is that of *bone quality*. The concept of bone quality was introduced in

response to the recognition that bone mass or density is an insufficient predictor of fracture risk.[40,41] While the term is somewhat loosely defined, bone quality comprises the parameters of bone discussed earlier, which illustrate or contribute to its mechanical integrity and, therefore, to the fracture risk in the patient or population. As well, measurements of bone quality typically include information about bone remodeling, which can be quantified using histomorphometric techniques. Remodeling contributes to both the architecture of the bone tissue and to the material properties of the bone (newly formed bone is only lightly mineralized; the degree of mineralization then increases gradually over time, eventually reaching a plateau). All of these parameters and their interrelationships are illustrated in Figure 1. Alterations to any one of the underlying factors can have profound effects on the overall mechanical properties and, therefore, the fracture risk of the patient. As it happens, fluoride can directly affect bone at all of these levels; they must all be considered in order to fully understand how fluoride alters the mechanical properties of bone.

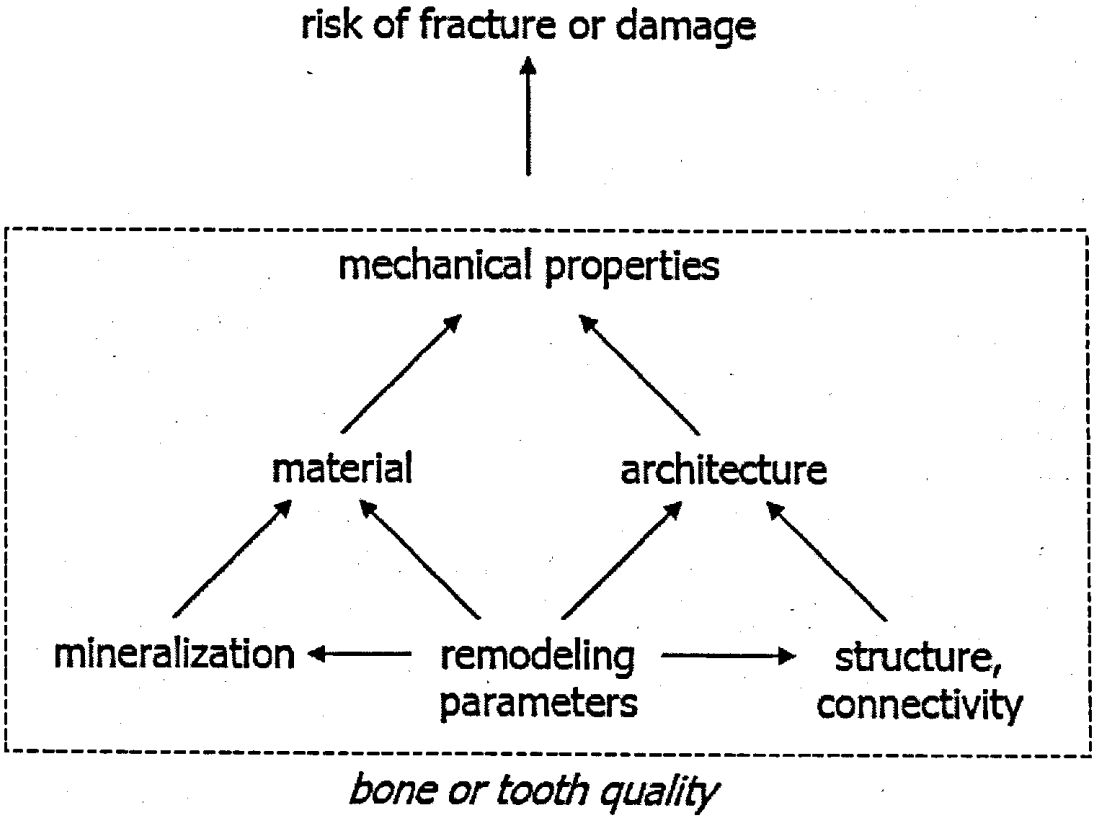

FIGURE 1. Bone and tooth quality. The overall properties of bone or teeth depend on both the composition and structure of the material (after Chachra[93]).

2. Fluoride and Bone Material

a. Effect on Bone Mineral

Regardless of concentration or dose, fluoride is incorporated into bone mineral during formation via a physicochemical mechanism. Fluoride substitutes for the hydroxyl group in hydroxyapatite, forming fluorapatite. This substitution, while no means complete (even in highly fluorotic bone, fluoride replaces only about a third of the hydroxyl ions),[42] nevertheless has profound consequences. The fluoride makes the crystal lattice more compact and stable,[38] and a mixture of fluorapatite and hydroxyapatite has been shown to be more stable against dissolution than either component individually.[43] The onset of mineralization is also delayed, resulting in increased osteoid formation.[44] The concentrations of other contaminant ions, such as carbonate and magnesium, appear to be affected as well, although the exact effect remains controversial.[38] Finally, fluoride shifts the mineralization profile of bone (a histogram of density fractions) towards denser, more mature fractions, and fluoride appears to be concentrated in these denser fractions.[44]

b. Effect on Bone Cells

While fluoride is passively incorporated into bone mineral at all concentrations, it appears to only affect bone cells at much higher serum levels than would be experienced through drinking fluoridated water alone.[35] It is well known that high levels of fluoride increase bone mass in patients subjected to fluoride therapy and in fluorotic individuals (discussed further below). This is a result of both the increased resistance to resorption of fluoridated bone mineral, as well as a mitogenic effect of these levels of fluoride on osteoblasts. This is supported by work both in vivo[42] and in culture.[45] However, the fluoride-affected osteoblasts appear to be flattened and moderately active rather than plump, cuboidal and highly secretory. This suggests that, while fluoride is mitogenic and a promoter of differentiation of osteoblast precursors, it is somewhat toxic to individual cells at these concentrations.[46] Nevertheless, the overall effect is of increased bone formation. While the effect of fluoride on osteoclasts is less well understood, there is some in vitro evidence that sodium fluoride decreases the number of resorption lacunae as well as the amount of bone resorbed per osteoclast.[47] The net result of these effects is an increase in bone mass, which accounts for the interest in fluoride as a therapy for osteoporosis.

c. Effect on Bone Architecture

Therapeutic administration of moderate doses of fluoride (tens of milligrams per day) to patients with osteoporosis results in a marked increase in bone mass. However, osteoporosis-associated bone loss results in loss of connectivity as well as thinning of the remaining trabeculae, and both are thought to be mechanically significant. At present, no biological pathway is known to restore the connectivity, and it is therefore thought that all types of therapy are similarly ineffective at doing so.[48] Accordingly, fluoride therapy has been shown to increase trabecular thickness, but leaves the connectivity unaltered. In addition, trabeculae, thus formed, appear to be resistant to perforation by resorption.[49] Rats who ingested fluoridated drinking water in a range of concentrations (0, 2, 4, 6 mM/L) displayed similar changes to cancellous bone, and the effects were found to be dose dependent.[50] Finally, biopsies obtained from individuals who received fluoride therapy for an average of 39 months displayed no change in the trabecular bone volume compared to normal controls, although this might be attributable to their greater age (68 ± 9 years vs. 55 ± 10 years). Parameters of bone formation (osteoid volume, surface, and width) were elevated, indicating the trabecular bone volume was increasing.[38]

d. Effect on Collagen-Mineral Interface

Studies that have assessed the effect of moderate to high doses of fluoride on the mechanical properties of bone have observed that, while the amount of bone present may be unchanged or increased, the mechanical strength declines.[51,52] Similarly, fluoride administration can result in increased mineralization, as assessed by density fractionation and microhardness measurements,[53] although this was not observed by backscattered electron imaging in a minipig model.[54,55] Increased mineralization would suggest that the strength and stiffness of the bone should also increase, which is not the case; this suggests that some other parameter must be altered by fluoride administration. A negative relationship has been observed between the bone mineral crystal width (measured by X-ray diffraction) and the femoral failure stress,[51] which suggested that the increased mineralization has a deleterious effect on the collagen-mineral interface; this is supported by small-angle X-ray scattering studies in both fluoride-treated humans[56] and minipigs.[54] The degradation of the interface has been attributed to the deposition of large, extrafibrillar mineral crystals,[54,56] which in-

crease the degree of mineralization of bone without contributing to the mechanical properties. It was also suggested[57] that mineral crystals formed during fluoride exposure (perhaps due to their increased size) do not bond as tightly to the collagen molecules, resulting in reduced interfacial strength compared to normal bone. Evidence to support this mechanism was provided by some recent work using atomic force microscopy to assess the effects of in vitro exposure to sodium fluoride on the collagen-mineral interface;[58] the interface between collagen and fluorapatite appears to be weaker than with the native hydroxyapatite. This in vitro treatment has also been shown to have a negative effect on mechanical properties.[59] It seems likely that the biological and physicochemical effects of fluoride on the collagen-mineral interface act synergistically to compromise the physical properties of bone material in highly fluoridated tissue.

3. Fluoride and the Mechanical Properties of Bone

As discussed above, fluoride has a complex, dose-dependent suite of effects on bone, including altering the amount of bone, the structure, and the mineral-collagen interface. These result in changes to the mechanical properties of bone, and by extension, to the fracture risk. Clinical techniques, such as dual-energy X-ray absorptiometry or histomorphometry, while useful, cannot assess these integrated factors. In order to quantify changes to mechanical function, one of two approaches must be used. One approach is epidemiological; the fracture rate in a given population (such as a clinical trial of patients receiving therapy for osteoporosis, or a region with fluoridated water) can be measured. The second approach, commonly used in animal studies, is to use in vitro mechanical testing techniques as a proxy for fracture risk. As discussed in detail below, the overall picture of the effect of fluoride on mechanical properties that has arisen, based primarily on animal studies, tentatively suggests a dose-dependent effect on mechanical properties: improvement with increasing fluoride content to an optimum point, followed by severely compromised mechanical properties at high concentrations of fluoride (fluorosis).

a. In Vitro Studies in Animal Models

The evidence that emerges from in vitro mechanical testing of bone from animal studies clearly indicates that high doses of fluoride are detrimental to mechanical properties. Controversy remains over whether

there is an optimum fluoride dose or concentration that leads to improved bone strength.

A study by Turner et al.[60] established a weak, biphasic response to fluoride ingestion in young (21 day old) rats. The rats were fed a low-fluoride diet, and their drinking water was fluoridated at 0, 1, 2, 4, 8, 16, 32, 64, or 128 ppm. The rats were sacrificed after 4 months. The fluoride content of the vertebrae was assessed, and femora were tested in three-point bending. It was found that the peak bone strength occurred with a fluoride intake of 16 ppm, corresponding to a bone fluoride content of 1216 ppm and decreasing thereafter. A segmented regression model was postulated to describe this biphasic relationship. This work was in agreement with an earlier study.[61] A later study by the same group[62] again began with 21-day-old rats, ingesting water fluoridated at 0, 5, 15, or 50 ppm, but followed them through maturity and senescence: 3, 6, 12, or 18 months. In this study, no positive effect of fluoride treatment was observed, although there was a negative effect at higher doses. No effect of fluoride was observed in a similar study[63] in which femora from rats exposed to drinking water fluoridated at 0, 25, 50, and 75 ppm were tested in torsion. However, the fluoride content of these bones (2026-11 716 ppm) was greater than the peak fluoride content observed by Turner et al.,[60] which may explain the discrepancy. Beary[64] found a similar decrease in bone strength in rat femora with high fluoride intake (> 45 ppm). Søgaard et al. [65] examined the mechanical properties of vertebrae (primarily cancellous rather than cortical bone). The rats were 3 months old at the start and ingested water fluoridated at 0, 100, and 150 ppm for 3 months before sacrifice, resulting in fluoride levels of 343, 3295, and 4617 ppm, respectively. While no changes to the failure load or stress were observed, the mechanical parameters corrected for ash content (a measurement of bone quality) were adversely affected. Similarly, Mosekilde et al. [66] found reduced trabecular bone strength in pigs with an average bone fluoride concentration of 2836 ppm. Finally, a study examining high doses of fluoride in rabbits showed compromised mechanical properties, despite increased bone mass, mineralization, and hardness.[51,53]

While animal models are useful in providing information about high doses of fluoride, which would be difficult to obtain in humans, there are two major caveats to be considered prior to extrapolating to the human case. The first is that the rate of fluoride incorporation in other species may not be the same as in humans. For example, in rats, the rate of fluoride incorporation is an order of magnitude less than in humans, suggesting that administration of water fluoridated at 1 ppm to humans

would be the equivalent of 10 ppm in rats.[60] The difference is partially accounted for by differences in intestinal absorption between rats and humans.[67] The second difference is that animal studies typically examine the effect of high doses of fluoride for short times, rather than the low doses and long exposures of humans. While low doses of fluoride are thought to act by altering bone at the level of crystal structure[38] and the mineral-collagen interface,[57] high doses also affect bone cells and, therefore, remodeling processes.

b. In Vitro Studies in Humans

Data correlating fluoride content and bone quality by in vitro mechanical testing in humans are scarce. Richards et al.[22] examined vertebral trabecular bone cylinders from individuals aged 20 to 91 years, who were not exposed to artificially fluoridated water, to elucidate changes in bone mechanics and fluoride content with age. They found that the bone mass and the bone strength, when normalized for the bone mass, decreased with increasing age. While the fluoride concentration increased with age, it did not affect the bone quality in a way that was independent of age and gender effects. At the other limit of fluoride exposure, an assessment of the bone quality of iliac bone biopsies from osteoporotic patients who had received fluoride therapy[65] indicated there was a reduction in bone strength and bone strength normalized for ash content, and no increase in bone mass, after 5 years of fluoride therapy of 40 to 60 mg/day.

c. Fluoride and Fracture Rate in Humans

Fluoride administration has been under investigation as a therapy for osteoporosis for four decades.[29] The primary advantage of fluoride is that it is one of the few therapies known to be anabolic; fluoride administration causes increased bone formation, rather than simply maintaining the amount of bone present. However, fluoride therapy has been complicated by a narrow therapeutic window and differences in response depending on the formulation of the administered fluoride.[68] Fluoride therapy, in conjunction with calcium and vitamin D, is unambiguously associated with increased axial bone mass;[69] however, its use remains controversial as some studies have reported no decrease in the rate of fracture[70,71] or associated lower limb pain,[72,73] which is likely caused by stress fractures.[74] These have been attributed to resorption of extant bone, the initial step in fluoride-induced bone remodeling, lowering the

bone mass and strength of severely osteoporotic individuals to below the fracture threshold.[75,76] However, studies with low-dose sustained release[77] or intermittent[78] fluoride administration suggest that it may be possible to reduce fracture incidence in osteoporotic patients without the associated side effects. The experience of fluoride as a therapy for osteoporosis highlights the complex, dose-dependent effect of fluoride on the mechanical properties of bone.

III.C. Municipal Water Fluoridation and Fracture Risk

Many municipalities fluoridate their drinking water. The U.S. Public Health Service issues recommendations, which are followed throughout the world, for the concentration of fluoride to be used. It varies from 0.7 to 1.2 ppm, depending on the average and maximum air temperatures, and is based on an empirical relationship between temperature and water consumption.[79–81]

Ingestion of water fluoridated at these levels of approximately 1 ppm is generally considered to have no detrimental side effects. However, long-term exposure to fluoride can result in significant fluoride accumulation in the skeleton, and concern remains that this may have adverse effects. In addition, it may result in changes to the structure or degree of mineralization of the bone (as is observed with short-term ingestion of higher doses of fluoride), and this may have mechanical consequences. This would, in turn, affect the incidence of osteoporosis, manifested as fracture rate.

Despite the large number of published studies, considerable controversy remains regarding the issue of fluoridated water and fracture risk, with studies indicating an increased, decreased, or unchanged risk of fracture with fluoridated water consumption. A complete review of such studies can be found elsewhere,[82] but a description of a number of studies will illustrate a wide variation in findings. For example, two studies correlating the regional variation of hip fracture in the United States[83] and in England[84] to local water fluoridation showed a slightly increased risk of fracture. Conversely, two studies comparing fractures rates in fluoridated and unfluoridated communities[85,86] found a reduced risk of fracture in the fluoridated communities. One study[87] found a reduced vertebral and hip fracture risk but an increased risk of wrist fracture. A similar study that focused on older women, who are at the highest risk for osteoporosis-associated bone fractures, found there was a slightly reduced risk of hip and vertebral fracture for women who were continuously exposed to optimally fluoridated water for two

decades compared to those who were not exposed.[88] Finally, a number of studies[89–91] showed no change in the risk of fracture as a result of water fluoridation.

While studies such as these must be interpreted with caution because of the limitations of retrospective epidemiological studies, two conclusions can be drawn from this mass of data. First, water fluoridation at 1 ppm most likely has no effect on bone mechanical properties, although it is impossible to exclude a small effect. This is confirmed by a meta-analysis that examined 29 studies to determine if a relationship existed between skeletal effects of fluoride and water fluoridation; no evidence for such a relationship was found.[92] Second, in order to determine if there is a 10% to 15% increase in fracture rate at an appropriate level of certainty would require more than 400 000 people enrolled in a cohort study—clearly prohibitive.[83] These findings were substantiated by a study to investigate the materials properties of femoral heads from residents of Toronto and Montreal, cities with and without municipal water fluoridation. Even a cursory glance at the data reveals that the fluoride content and the strength of bone are highly variable (Figure 2). This suggests that any potential effects of municipal water fluoridation at 1 ppm on the mechanical properties of bone, whether positive or negative, are not large enough to be observable in light of the large variation in fluoride incorporation and physical characteristics in a normal human population.[93] The absence of negative effects on bone, coupled with the well-documented reduction in the incidence of dental caries, suggests that the public health effect of municipal water fluoridation is a net benefit.

There is one caveat, however. Skeletal fluorosis is endemic in regions where natural water supplies contained levels of 10 to 20 ppm of fluoride, such as in some parts of India,[94] as discussed further below. However, there is some evidence that individuals living in areas where the water fluoride content is as low as 4 ppm may have asymptomatic skeletal fluorosis and are at an increased risk of bone fracture compared to individuals in areas with water fluoridated at 1 ppm.[95] This is the upper limit permitted in drinking water, and there are some areas in North America where the water fluoride levels are this high.[96]

III.D. Skeletal Fluorosis

At chronically high levels of fluoride exposure, a markedly deleterious effect on the skeleton is observed. These changes are termed *skeletal fluorosis.* Exposure to excessive fluoride can occur through a number

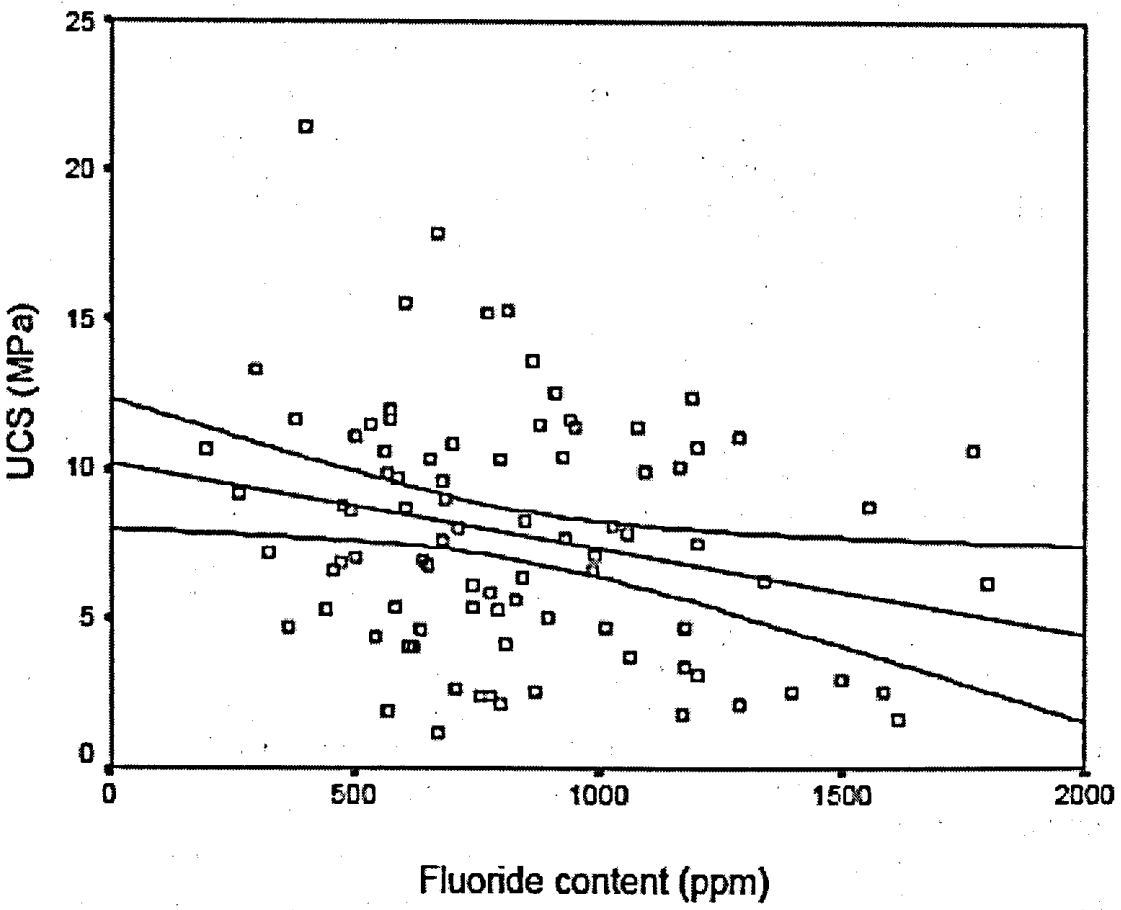

FIGURE 2. Variation in fluoride content and strength of bone. Both the fluoride content and the strength (ultimate compressive stress [UCS]) of trabecular bone from human samples are widely variable. While there is a significant relationship between them ($p < 0.05$), less than 5% of the variation in the strength is explained by the fluoride content ($R^2 = 0.048$). This may also be a result of weakly increasing fluoride content or loss of bone with age.[93]

of routes, three of which are the most important. Hydric fluorosis can result from either prolonged or sporadic exposure to water with a greater-than-optimal fluoride concentration, as has been observed in certain areas of India,[94,97] or as a result of consumption of Vichy Saint-Yorre mineral water.[26] Industrial fluorosis has been observed in workers in a number of industries that involve fluoride processing. Finally, iatrogenic fluorosis has been observed in individuals who have received fluoride as a therapy for osteoporosis or fluoride-containing drugs.[14]

As with dental fluorosis, discussed below, there appears to be a strong genetic component to susceptibility to skeletal fluorosis; even at high concentrations of fluoride in the drinking water (1.2–8.9 ppm), the

incidence of skeletal fluorosis was approximately 40%, and it varied with not just the concentration but also the population.[97] Similarly, roughly one-third of patients given fluoride as a therapy for osteoporosis did not show an effect of the therapy.[98] The prevalence of nonresponders suggests a genetic determinant of susceptibility to fluoride.

Fluorosis is usually clinically identified by observation of increased radiological density of bone (osteosclerosis), particularly of the spine. At higher exposures, ectopic calcification of soft tissues, such as ligaments and capsules, is also observed.[14] The former symptoms become apparent at fluoride concentrations of about 4000 ppm (in dry bone) and is associated with long-term ingestion of excessively fluoridated drinking water. The latter symptoms, associated with joint pain and immobilization, usually result from prolonged intake of 20 to 80 mg/day of fluoride.[99] Observed fluoride contents in fluorotic patients range from about 0.56% to 1.33% of ash weight.[100]

Skeletal fluorosis is characterized by a number of changes to the architecture, remodeling, mineralization, and mechanical properties of bone. Histomorphometry of transiliac biopsies from fluorotic patients shows profound differences compared to controls. They include increased cancellous bone volume (which is consistent with the radiologically observed osteosclerosis) as well as increases in cortical bone width and porosity.[14,42,101]

Remodeling in fluorotic bone is strongly tilted in favor of formation, characterized by increased osteoid (newly formed bone) parameters including perimeter, width, and volume. In patients doubly labeled with tetracycline (which binds to bone and is visible under UV, allowing the bone formation between the two injections to be quantified and a rate determined), the mineral apposition rate decreased, and the mineralization lag time increased, which contributed to the increased osteoid.[14,42,94] In a rat model of high fluoride intake combined with renal insufficiency (thereby exacerbating the effect of the fluoride), the osteoid volume was increased 20-fold compared to controls; the wide osteoid seams were characteristic of osteomalacia.[102]

Increased fluoride concentration in bone is associated with increased mineralization in fluoride-treated humans,[103] animal models,[53] and fluorotic bone.[104] However, in skeletal fluorosis, the increased mineralization is associated with characteristic defects. These include enlarged lacunae and circumlacunar mineralization defects ("mottled osteons")[101,104,105] and linear formation defects in cancellous bone.[106]

Finally, animal models of skeletal fluorosis indicate that, despite increased mineralization, excessive fluoride exposure is associated with

decreased mechanical integrity.[51,66] Similar decreases in mechanical properties have been observed for some patients administered fluoride as a therapy for osteoporosis.[65]

III.E. Summary

The wide-ranging effects of fluoride on the materials science of bone have been extensively researched. As was shown earlier, the response of bone to fluoride is highly dose-dependent. As well, fluoride can affect virtually every level of the hierarchical structure, from the properties of the mineral to the interface to the ratio of mineral to protein (degree of mineralization) to the macroscopic structure of the bone. Accordingly, the effect of fluoride on the mechanical properties of bone is similarly wide ranging. As the mechanical properties of bone are of considerable clinical and public health significance, increasing our understanding of the effect of fluoride will aid us in designing both public health interventions and therapies for osteoporosis.

IV. FLUORIDE AND TEETH

IV.A. Tooth Biology

1. The Composition and Structure of Teeth

The outer layer of teeth consists of hard, highly mineralized *enamel.* The hardest material in the body, the enamel provides a surface against which to chew food. However, the enamel is quite brittle; it is supported by an underlying region of *dentin* which, like bone, is a composite of collagen and mineral. Dentin is not as hard as enamel, but it is considerably more resilient. The central part of the tooth is occupied by the *pulp,* a vascularized, innervated structure. Below the gum line, the outer layer of the tooth is termed the *cementum.*

a. Enamel

The basic structure of enamel is the *enamel rod,* a rough cylinder of material, between 4 and 8 μm in diameter, which is approximately perpendicular to both the surface of the dentin and the surface of the tooth. These rods pack together laterally to form the solid enamel surface. Enamel is the most mineralized tissue in the body, consisting of 96% mineral and 4% organic material and water. As with bone, the mineral

is predominantly hydroxyapatite.[107] The long axes of the hydroxyapatite crystals are generally oriented parallel to the longitudinal axis of the enamel rods. While the enamel seems smooth and sealed, this microstructure allows ions to diffuse into the tissue.[108]

With its extremely high mineral content, enamel is essentially a ceramic. Like most other ceramics, it is extremely hard (enamel has a hardness comparable to mild steel), which makes it highly resistant to wear during mastication. The trade-off in properties is that it is notably brittle. The enamel is, therefore, supported by an underlying region of a tougher material, dentin, which is less mineralized and more resilient.

a. Dentin

Dentin is not a homogeneous material. The outer layer, just underneath the enamel, is termed the *mantle dentin* and is approximately 150 μm thick. This region is characterized by a relatively high percentage of organic matrix, resulting in a softer tissue.[109] The collagen in the mantle dentin appears as very distinct large-diameter fibrils. The *circumpulpal dentin*, found between the mantle dentin and the pulp, has a tubular microstructure. The tubules are oriented in the enamel-pulp direction. The collagen in this region aggregates into smaller fibrils and aligns itself at right angles to the tubules.[110] The mineralized intertubular area constitutes the main part of the dentin and is called, appropriately enough, *intertubular dentin.*[111] The tubules are surrounded by and lined with a 1 μm thick layer of highly mineralized tissue, known as the *peritubular dentin.*[110,112] The function of the peritubular dentin is not well established, but it is believed to enhance the stiffness of dentin.[113] The amount of intertubular dentin decreases towards the pulp.[111] Conversely, the number of tubules per unit area also varies depending on the location within the dentin and increases closer to the pulp.[114] Finally, the size of the dentinal tubules depends on their location within the dentin and on the age of the tooth.[111] The size may also vary with other conditions, such as the mineralization status of the tooth or the fluoride content.[115]

Dentin has a characteristic ultrastructure consisting of a hydrated matrix of type I collagen, which is reinforced with a nanocrystalline carbonated apatite. Approximately 70% of the dentin by weight (50% by volume) is mineral, primarily hydroxyapatite. The collagen and noncollagenous proteins make up approximately 18% of the weight (24% by volume), and the remainder is water.[111] However, the composi-

tion does vary by dentin location, resulting in site-specific mechanical properties. The primary mechanical function of the dentin is to support the enamel when the dentin-enamel complex is subjected to mechanical loading.[107] This needs to both absorb mechanical energy and transmit it to the maxilla and mandible accounts for the structure of the tooth interior. The more compliant mantle dentin, directly beneath the dentin-enamel junction, deforms to a greater degree than the circumpulpal dentin.[116-118] As well, the peritubular dentin, with its very low organic content, is a much stiffer material that reinforces the intertubular dentin.[112]

2. Development of Dental Tissues

Dental tissues are derived from two embryological sources. The enamel is of epithelial (ectodermal) origin, while the dentin, cementum, pulp, and periodontal ligament are of mesenchymal (mesodermal) origin.[109]

Dentin is formed by two simultaneous processes. Cells known as *odontoblasts* secrete a collagenous matrix, and they also mediate the formation of mineral crystals in close apposition to this matrix.[119] The tissue is mineralized by calcospheric calcification, which involves the deposition of crystals in discrete areas of the matrix. At these mineralization foci, calcospherites—globular masses of crystal—form.[111] With continued growth, the calcospherites enlarge and then fuse into a single calcified mass,[110] resulting in fully mineralized dentin. Shortly after dentin formation is initiated, a number of distinct and almost simultaneous morphologic changes associated with the onset of amelogenesis (enamel formation) occur in the developing tooth. The cells of the internal dental epithelium, now ameloblasts, begin to secrete the enamel matrix, which immediately becomes partially mineralized. As this first increment of enamel is formed, the ameloblasts begin to move away from the dentin surface. When secretion of the full thickness of enamel is complete, the ameloblasts pass through a brief transitional phase during which significant morphologic changes occur. The postsecretory ameloblasts shorten slightly and become involved in enamel maturation. A cyclical process then begins: alternate bursts of activity selectively remove water and organic material, while additional inorganic material (calcium and phosphate ions) is introduced. The cyclical nature of this process is reflected in ameloblast morphology, with the cells alternating between possessing a ruffled border, associated with the introduction of inorganic material, and possessing a smooth border, associated with the removal of protein and water from the enamel matrix.[120]

3. Remodeling of Dental Tissues

While bone tissues are constantly remodeling to alleviate the damage that accumulates during normal mechanical loading, dental tissues remodel primarily in response to their chemical environment. The mineral component of teeth is a defect-containing carbonated hydroxyapatite with a complex crystal structure that is readily dissolved in acid.[121,122] In the presence of cariogenic (cavity-causing) bacteria, including fermentable carbohydrates and saliva, the mineral of the enamel undergoes cycles of demineralization and remineralization.[123] The changes in the mineral structure of the enamel depend on the equilibrium between *demineralization* and *remineralization* processes.[124] The process that is occurring at a given moment is determined by the balance between pathological factors and protective factors in the dental biofilm.[125-127] Dental caries, of course, manifest when this dynamic process is skewed in favor of demineralization. Conversely, one of the major functions of saliva is to help defend dental tissues. Saliva is a saturated solution of calcium (in a soluble complexed state) and phosphate, which inhibits demineralization and fosters remineralization, and also contains buffering acids. In addition to this physicochemical role of saliva, certain of its constituents are antibacterial, and the salivary proteins play a role in reversing or arresting the caries process.[128,129]

Unlike enamel, dentin can only be demineralized in pathological circumstances as a result of exposure to oral fluids—for example, when the protective enamel layer is removed by dental caries or abrasion. However, it is not a completely static tissue. The dentin that is initially present in the newly formed tooth is known as primary dentin; secondary dentin is deposited during the lifespan of the tooth and is responsible for the gradual shrinkage of the pulp. Tertiary dentin, on the other hand, is formed in response to pulp and tooth irritants and is characterized by a thickening of the peritubular dentin, which obliterates the dentin tubule structure.[111]

4. Incorporation of Fluoride into Dentin and Enamel

For the purposes of caries prevention, fluoride can either be administered systemically (by ingestion, such as fluoridated water) or topically (fluoridated toothpaste, fluoride preparations applied by a dentist). Systemic fluoride is incorporated into teeth during formation; for the primary (baby) teeth, this is primarily after birth, and for the permanent dentition (adult teeth), this is during the first years of life. Systemic fluoride

incorporation, therefore, results in fluoride throughout both the dentin and enamel. Topical fluoride, on the other hand, is only incorporated into the most superficial layer of enamel and, therefore, has very little influence on the rest of the tooth structure or properties.

The diffusion of fluoride ions into the enamel during exposure to topical fluoride (such as toothpaste) occurs during the demineralization and remineralization process described above. The fluoride ions can bond more strongly to the calcium atoms than the hydroxyl (OH^-) ion, and fluoride ions, therefore, readily replace hydroxyl ions in enamel.[130] The fluoride ion occupies a different lattice position than the hydroxyl ion. As it is located in the center of a triangular array of calcium ions, the system is symmetric and does not form a polar moment, as can occur with a hydroxyl ion. This results in increased stability of the apatite crystal and alters the kinetics of precipitation and dissolution.[38] The enamel is less susceptible to dissolution by bacterial acids; this is one of the mechanisms by which fluoride administration reduces the incidence of dental caries.

The apatite crystallites in the dentin are smaller than in the enamel and, therefore, have a higher surface area-to-volume ratio. They are composed of a poorly crystalline and highly substituted apatite with a lower calcium content and higher carbonate content. This results in higher solubility and a greater susceptibility to ionic substitutions of fluoride for hydroxyl.[131] The observed higher fluoride concentrations in dentin, compared to enamel,[132] are likely to be a result of these differences in crystal size and composition.

Despite their higher fluoride content, the crystallite size in dentin did not vary with fluoride content in teeth from populations exposed to different levels of drinking water. This contrasts with the enamel in which the length and width of crystallites tended to be greater in teeth coming from areas fluoridated at 1 ppm than those from areas with lower levels of water fluoridation.[133] A number of noncollagenous proteins, including phosphophoryn, bone sialoprotein, osteocalcin, osteonectin, and osteopontin, are found in mineralized tissues and are known to regulate mineralization,[134] and these data correlate previous findings that the mineralization of dentin is more closely regulated by proteins than that of enamel.[110]

IV.B. Dental Fluorosis

The impact of fluoride on the materials science of bone has been studied over a wide range of fluoride exposures and contents, as discussed

above. In contrast, studies of dental tissues have largely focused on the caries resistance of the surface, and relatively little research has been performed to evaluate the effects of low doses of fluoride on the physical properties of dental tissue. What little research there is into the materials science of dental tissues has largely focused on changes effected by high exposures to fluoride, which results in dental fluorosis, a disease which is largely characterized in terms of aesthetic and functional changes to the dental tissue. We, therefore, begin with an overview of dental fluorosis before we discuss the effects of fluoride exposure on the structure, composition, and mechanical properties of bone.

1. Definition and Prevalence of Dental Fluorosis

Dental fluorosis is the only known side effect of systemic fluoride use in the doses utilized for caries prevention in North America.[81] Dental fluorosis is defined as hypoplasia and/or hypomineralization of tooth enamel or dentin produced by chronic ingestion of excessive amounts of fluoride during the period of tooth development.[135,136] Evidence of dental fluorosis can range from the occurrence of thin, white, opaque lines running across the tooth surface (mild fluorosis, normally only seen when tooth is dry) to loss of the major part of the outer enamel, resulting in a change in the anatomical shape of the surface/tooth (severe fluorosis).[137]

While dental fluorosis primarily affects enamel during formation, it can also affect dentin. All damage occurs before the eruption of the teeth; however, the appearance of the enamel may be altered after eruption. The damage caused by abrasion and extrinsic staining is not related to posteruptive presence or absence of fluoride.[138] Rather, the brownish-black discoloration observed with severe fluorotic defects is a secondary phenomenon, resulting from the increased susceptibility of the porous surface of severely mottled areas to staining.[139]

The time of exposure to elevated fluoride levels and the duration of enamel mineralization determines the incidence, oral distribution, and severity of dental fluorosis.[137] This means that the earlier in life the body is exposed to elevated fluoride levels, the greater the risk of developing dental fluorosis. The distribution of dental fluorosis in the dentition is governed by the duration of mineralization (time elapsed between the initiation of mineralization and its completion) and the time of eruption; the later the enamel mineralization occurs, the more severe dental fluorosis can be expected. The incisors and the first molars erupt first, followed by the second molars and the canines, and finally the premolars and third molars (wisdom teeth); the premolars tend to display the

highest incidence and severity of dental fluorosis. In contrast to bone, the mechanisms by which fluoride affects developing enamel at these high doses are not well understood. A number of mechanisms have been proposed to explain the formation of fluorosed enamel: a systemic effect of fluoride on calcium homeostasis, altered matrix biosynthesis (protein secretion, synthesis of mineral composition), a direct or indirect effect on matrix proteinases affecting protein removal, or specific effects on cell metabolism and function.[140] Most of the available evidence suggests that fluoride has an effect on cell function, either directly through interactions with the developing ameloblasts or more indirectly by interacting with the extracellular matrix.[137]

When municipal water fluoridation was introduced, fluoride in the range of 1 ppm was known to result in about 10% of the population developing very mild fluorosis.[141] However, more recent studies show evidence of a higher prevalence of dental fluorosis in the community,[92] which may be due to an increase in the sources of fluoride (e.g., toothpaste, mouthwashes). For regions with water fluoridated at the optimal level of approximately 1 ppm, an average prevalence of dental fluorosis of 48% was observed, with the incidence of fluorosis severe enough to be "of aesthetic concern" as high as 12.5%. [92] Reports published in the 1980s corroborate this increase in fluorosis, which was observed in communities with and without municipal water fluoridation.[4,142] While these increases in dental fluorosis were typically confined to the mild and very mild end of the scale,[143] the increased prevalence does imply a total fluoride ingestion in excess of that occurring during the 1930s.[144]

As the severity of dental fluorosis increases, the incidence of caries decreases until the destructive forms of fluorosis become prevalent. Under the latter conditions, the loss of enamel integrity and exposure of underlying dentin results in an increase in caries. However, even when the tooth integrity is compromised, the high-fluoride exposure means that the lesions usually progress slowly and frequently become arrested.[145] The caries incidence in children living in an area containing 5.8 ppm of fluoride in the water supply, for example, is twice that of children living in an area containing 3.5 ppm of fluoride (2.8 teeth decayed, missing, or filled per person at 5.8 ppm, vs. 1.4 per person at 3.5 ppm), but the incidence is still considerably higher for children living in low F areas (0.1-0.2 ppm).[146] However, the frail condition of the enamel in severely fluorotic teeth makes it very brittle and it is, therefore, extremely difficult to provide effective restorations (fillings) when cavities do occur.[147,148] As with the response of bone tissue to fluoride exposure, individuals seem to vary in their genetic susceptibility to

dental fluorosis, both in terms of the appearance and the changes to the physical properties of teeth.[149]

2. The Relationship Between Fluoride Concentration and the Severity of Dental Fluorosis

While dental fluorosis does not exist in the absence of fluoride,[140,150] a number of studies have shown that the severity of dental fluorosis does not correlate well with tooth fluoride content.[132,151–153] As the prevalence and incidence of dental fluorosis increases in fluoridated areas,[4] this lack of a strong correlation is counterintuitive.

As discussed in Section II, the amount of fluoride an individual ingests is dependent on the amount of fluoride he or she is exposed to in the environment. As calcified tissues are the main storage area for ingested fluoride,[5] the amount of fluoride found in calcified tissues should be related to the amount of fluoride ingested. However, the amount of fluoride that is sequestered in the teeth will be related to not just the exposure but also the metabolism of the individual, particularly the effectiveness of renal clearance. This individual response to fluoride exposure may account for some of the variation in the severity of dental fluorosis.

As well, a number of studies, in animals and in humans, have shown that the fluoride concentration in teeth varied over a wide and overlapping range for each level of severity of dental fluorosis.[132,151–153] As the severity of dental fluorosis cannot be entirely explained by fluoride concentration in the teeth, this suggests that other factors contribute to the incidence and severity of fluorosis. Several epidemiological studies have shown that susceptibility to dental fluorosis may vary by ethnicity.[154–156] Similarly, a laboratory study of mice demonstrated that some strains of mice are more susceptible to dental fluorosis than others.[157] Based on these findings, it can be inferred that the incidence and severity of dental fluorosis is related to the genetic susceptibility of individuals. As a result of this range of individual response (both genetic and physiological), the mechanical properties of fluorotic teeth do not correlate strongly with the fluoride content.

IV.C. Fluoride and the Materials Science of Teeth

1. Overview

As with bone tissue, the mechanical function of teeth depends on the composition (the degree and distribution of mineralization), the mac-

roscopic structure, and the micro- and ultrastructural organization of the constituents. By analogy to the term *bone quality,* described above, these parameters, which contribute to the mechanical properties, are collectively described as *tooth quality* (Figure 1). Tooth quality relates to the ability of the tooth to fulfill its function of sustaining mastication forces, and it can be evaluated by measuring the tooth's mechanical, material, and structural properties.

Unlike bone, teeth comprise two distinct mineralized tissues: enamel and dentin. Enamel is a challenging material to investigate, as it is extremely brittle, and it is very difficult to dissect it away from the underlying dentin. However, it is amenable to some studies of material and mechanical properties, including S-ray diffraction to determine crystal size and microhardness testing to quantify the mechanical properties. Dentin can be analyzed using some additional techniques for the bulk material, including assessment of mineralization, determination of ultrasound velocity (a proxy for elastic modulus), and characterization of the size and structure of the tubules.

In this section, data from a number of studies will be presented. These studies investigated the material, structural, and physical properties of dental tissues and correlated them with the fluoride content or severity of dental fluorosis.[115,132,149,158–161] The fluoride content of tissues was measured using instrumental neutron activation analysis.[162] The severity of dental fluorosis was assessed using one of two methods. The Thylstrup and Fejerskov Index (TFI) was used to describe the clinical appearance of dental fluorosis.[163] A 10-point scale is used in which TF0 represents no observable dental fluorosis and TF9 is the most severe case. The second method used was quantitative light-induced fluorescence (QLF), which is a dental diagnostic tool that has recently been shown to be useful in the detection and quantification of dental fluorosis.[157] With this technique, fluorotic regions of the enamel surface appear bright in images. The degree of brightness and the area affected can be quantified using image analysis, which in turn allows the severity of dental fluorosis to be quantified.

2. Dentin and Fluoride Content

Analyses of human teeth from three cities with a range of water fluoridation (0.2, 0.7, and 1 ppm) provided data on the physical properties of the dentin. The samples used were third molars (wisdom teeth) that had been removed at surgery. The fluoride content of the dentin ranged from 110 to 860 ppm, and the severity of dental fluorosis ranged from

TF0 to TF4 (as unerupted teeth cannot exceed TF4 on this scale, this is the full range of severity).

The degree of mineralization of the dentin (the relative proportions of the mineral and the organic components), as measured by backscattered electron imaging,[103] did not vary with the concentration of the fluoride in the dentin (although there was some evidence of a correlation with the fluoride concentration in the enamel). Similarly, the microhardness of the dentin (which is closely related to the mineralization) did not correlate with the fluoride content of the dentin. However, there did appear to be a relationship between the TF score and the differential in hardness between the mantle dentin and the bulk dentin.[160] As mentioned above, no evidence of a relationship between crystallite size (measured by X-ray diffraction) and fluoride content was observed.[133]

In contrast to the limited evidence of a relationship between the composition and ultrastructure of the dentin and the fluoride content, image analysis of tooth sections reveals that the width of the dentin tubules increases with increasing fluoride content, although the amount of variability explained by the fluoride content is very low ($p < 0.005$; $r^2 = 7.3\%$).[115] As well, the ultrasound velocity (which is related to the elastic modulus, or stiffness) of the dentin was negatively correlated with the fluoride content of the dentin, although again the coefficients of determination were very low (9.5 to 10.2%, depending on direction). As the severity of dental fluorosis (by TF score) correlated with the dentin fluoride content, it is unsurprising that the width of the dental tubules and the TF score were also correlated. However, the ultrasound velocity appeared to increase with increasing TF score (positive correlation). This is at odds with the observation that the TF score is positively correlated with the dentin fluoride content ($p < 0.0005$) and the negative relationship between velocity and fluoride content.[115] While this discrepancy may be partly a result of the large amount of scatter in the data, it does point to the need for additional research into the relationship between tooth quality and fluoride content.

One way of limiting the variability in response observed in human populations is to study an animal model in which the test subjects are much more genetically homogeneous. As with the studies of bone tissue described earlier, the use of animal models also allows the investigation of much higher doses of fluoride than would be possible with a human population. An investigation utilizing three strains of mice, ingesting drinking water at 0, 25, 50, and 100 ppm, was used to determine the relationship of tooth quality to fluoride over a wider range of exposure and concentrations. In these mice, the dentin microhardness and min-

eralization correlated negatively with the fluoride content. The microhardness, but not the mineralization, also was significantly correlated with the severity of dental fluorosis (measured by QLF).[149] The severity of dental fluorosis varied by mouse strain, confirming earlier work that genetic susceptibility plays a large role in determining the response to fluoride exposure.[132,157]

3. Enamel and Fluoride Content

Unsurprisingly, given its different composition, structure, and development, the response of enamel to fluoride is quite different than dentin. As discussed earlier, it is extremely difficult to isolate enamel, which limits the methods that can be used to assess the response of the tissue to fluoride exposure.

The properties of the enamel of extracted wisdom teeth from individuals residing in areas with 0.2, 0.7, and 1 ppm of fluoride in their drinking water were also determined.[133] The fluoride content of the enamel ranged from 39 to 550 ppm. While the size of the crystallites in the enamel did not correlate with the fluoride content of the enamel itself, the size did correlate positively with the fluoride content of the dentin ($p < 0.001$ for both length and width). This is in striking contrast to the absence of an observed relationship between the crystallite size in dentin and the fluoride content, as discussed above. These data suggest that the crystal size in the dentin may be more closely regulated than in the enamel and, therefore, less responsive to fluoride.[133]

Returning to a mouse model of fluoride exposure, the microhardness of the enamel was also negatively correlated with the fluoride content (as was observed for the dentin). The microhardness also was negatively correlated with the severity of dental fluorosis.[149] The coefficient of determination values for the relationship between enamel microhardness and fluoride is much higher than that for dentin microhardness; this suggests that the enamel is more affected by fluoride exposure than the underlying dentin.[149]

IV.D. Summary

While the relationship between the materials science of bone and the fluoride concentration has been extensively studied, the study of a similar relationship for dental tissues remains in the very early stages, as is apparent from the limited number of studies and research groups referenced here. Nevertheless, it is clear that, as with bone, the response of

teeth to fluoride exposure is highly variable, both in terms of the severity of dental fluorosis and in terms of the tooth quality. This remains an area of considerable public health importance, and additional studies are required to further characterize the physical properties of teeth.

V. CONCLUDING REMARKS

As should be clear from this review, fluoride has a very broad suite of effects on mineralized tissues, from the atomic scale up to the macroscopic level, and the response of the tissues to fluoride is sensitively dose dependent. As well, individuals respond to fluoride to widely varying degrees, likely due to differing genetic susceptibilities. In any population, therefore, there is likely to be a broad range of responses to fluoride administration, which makes assessing the effects of fluoride particularly complicated.

Nevertheless, understanding the impact of fluoride on the physical properties of mineralized tissue has considerable clinical and public health importance. Water sources that are naturally fluoridated at high levels can have significant health impacts on communities. After four decades, public water fluoridation is widely considered to be a highly successful public health initiative, and it remains the single most effective method of reducing dental caries in a population, particularly in developing countries. Nevertheless, it is important to monitor the long-term effects of these low levels of fluoride in order to continue to make informed public policy decisions about its use. Finally, moderate doses of fluoride have been under investigation as an anabolic therapy for osteoporosis. An understanding of the interaction between fluoride and mineralized tissues will, therefore, enable us to make better public health and therapeutic decisions.

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