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Fluoride and Biological Calcification I: Effect of Fluoride on Collagen-Induced In Vitro Mineralization and Demineralization Reactions

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Abstract

An in vitro system employing collagen isolated from the sheep tendons to induce mineralization and demineralization reactions was used not only to study the effect of various concentrations of fluoride on the collagen-induced mineralization and demineralization reactions but also to compare their action with the inhibitors of mineralization and/or demineralization. Studies demonstrated that under physiological conditions, at lower concentrations (5×10^{-6} to 5×10^{-5} M) fluoride inhibited while at higher concentrations ($> 10^{-4}$ M), it stimulated the collagen-induced in vitro mineralization. At higher concentrations, fluoride was also found to inhibit the demineralization of the collagen bound preformed mineral phase. At low concentrations, fluoride acted like Mg^{2+} to inhibit mineralization while at higher concentration, it acted like crystal poisons (e.g., pyrophosphate phosphonates, citrate) to inhibit demineralization. However, unlike magnesium and pyrophosphate, fluoride at its higher concentrations was found to stimulate rather than inhibit the process of mineralization.

Keywords Collagen · Mineralization · Demineralization · Nucleation · Mineral phase · Hydroxyapatite · Fluorapatite

Introduction

Collagen isolated from different tissues (tendons, aorta, skin, etc.) has been employed in various in vitro studies as a model system to understand not only the mechanism of mineralization occurring in bones and teeth but also the mechanism of action of various inhibitors or promoters involved in controlling the mineralization of collagen-containing tissues under physiological and/or pathological conditions [1–5]. These studies have revealed that collagen acts as an enzyme (by lowering the activation energy) to catalyze the uptake of calcium and phosphate ions from the stable solutions to form matrix bound mineral phase, resembling hydroxyapatite in nature. The mineral phase thus formed gets tightly associated with the specific sites of the collagen. Studies have further demonstrated that, depending upon the saturation status of the reaction system/media, the collagen bound mineral phase can undergo either further growth or demineralization (release of calcium and phosphate ions from the matrix bound mineral phase into the soluble phase/reaction system). The calcium and phosphate ions of the matrix bound mineral phase have also been demonstrated to participate in iso-/hetero-ionic exchange reactions with the ions present in the soluble phase [6–8].

The association of the mineral phase with collagen (mineralization) has been shown to occur by a step-wise process. The calcium and phosphate ions first get bound at specific sites/groups on collagen before getting converted to the final form of mineral phase resembling hydroxyapatite in nature. From time to time, various biomolecules present in the body fluids, by acting either as inhibitors or promoters, have been postulated to be involved in the control of biological mineralization, both under physiological and pathological conditions. Depending upon their chemical nature, these biomolecules have been shown to act either by influencing a step involved in initiating the process of mineralization (e.g., Mg^{2+}, Zn^{2+}, Sr^{2+} phosphonoacetates) or act like crystal poisons by binding to the matrix bound mineral phase (e.g., pyrophosphate, citrate, phosphonates, polypeptides, mucopolysaccharides). It has been

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further shown that those biomolecules which act by influencing a step involved in initiating the process of mineralization do not have any effect upon either the ion exchange or demineralization reactions, while those which act like crystal poisons influence all the three reactions, i.e., mineralization, demineralization, and the ion exchange [7–15].

Fluoride is a normal constituent of all mineralized tissue including bones and teeth and its concentration in these tissues depends upon age and its amount ingested. Normal fluoride concentration in human serum and saliva has been reported to be 5–8 and 5–15 μM respectively [16]. Fluoride concentration in human body fluids is greatly influenced by the dietary status. Approximately, 35–50% of absorbed fluoride is taken by the skeletal tissues [17, 18]. Dental caries (tooth decay) is a widespread problem, affecting people all over the world. Fluoride through toothpaste, water, milk, mouth rinses, tooth gels, etc. is being routinely prescribed to prevent dental caries. The nature of the fluoride mostly present in the toothpaste is either sodium fluoride (NaF) or sodium monofluorophosphate (MFP). Fluoride is often referred to as a tooth toughener [19, 20]. High concentration of fluoride is also known to cause skeletal fluorosis [21]. Although fluoride uptake by the mineralized tissues is known to cause highly significant changes in their physical and chemical properties, the mechanism by which it acts is not clearly understood [22].

For the present study, in vitro system of mineralization, employing collagen isolated from the sheep tendons was used to not only investigate the effect of various concentrations of fluoride on the collagen-induced mineralization and demineralization reactions but also to compare the results thus obtained with the actions of magnesium and pyrophosphate on the above two processes.

Material and Methods

In Vitro Assay System to Study Mineralization and Demineralization Reactions Collagen employed during the studies was isolated from sheep tendon by the method of Thomas and Tomita as modified by Jethi and Wadkins [7, 8]. One hundred milligrams of collagen fibers was incubated at 37 °C in the assay system consisting of 87.5 mM Tris–HCl buffer (pH 7.4), 105 mM NaCl, 1.3 mM $CaCl_2$, and 1.2 mM KH_2PO_4 in a final volume of 25 ml. The final volume was made by using glass distilled water. Aliquots removed at different time intervals were filtered to determine the concentration of calcium and phosphate ions. Calcium or phosphate uptake, induced by the collagen to form matrix bound mineral phase, taking place at any given time was determined by subtracting its concentration present in the samples removed at that time interval from its value present in the samples removed at the start of incubation.

To study the demineralization of the preformed matrix bound mineral phase, pre-mineralized collagen preparations were obtained by incubating the known amounts of collagen fibers in the reaction system given above for 24 h. The mineralized matrices thus prepared were washed twice with Tris–HCl buffer before resuspending in the fresh incubation media in the absence of calcium and phosphate ions. The concentrations of calcium and phosphate ions present in the samples removed at a given time represented the extent of demineralization (release of ions) which has taken place at that particular time. Concentrations of calcium and phosphate in the samples obtained at different time intervals were determined by the methods of Baginski, Marie, Clark, and Zak [23] and Amador and Urban [24] respectively.

To study the effect of fluoride, magnesium, and pyrophosphate alone or in various combinations, on mineralization and demineralization reactions, after adjusting the pH of their solutions to 7.4, various amounts of their solutions were added in the reaction systems to give the desired concentrations. Percent inhibition or stimulation caused by a specific amount was calculated with respect to the control system where identical amounts of glass distilled water were added.

Statistical Analysis All values given in tables and figures are mean of five replicates. Statistical significance of the results thus obtained was determined by subjecting the data to one-way ANOVA with Tukey's test for mean comparison.

Results

In vitro studies employing collagen isolated from the flexor tendons of sheep were used not only to investigate the effect of various concentrations of fluoride on the collagen-induced mineralization and demineralization reactions but also to compare its mechanism of action with magnesium and pyrophosphate, the inhibitors known to be involved in the control of biological mineralization.

Studies on Mineralization: Uptake of Calcium and Phosphate Ions, Induced by Collagen and the Effect of Fluoride on It Results (Fig. 1) demonstrate that when 100 mg of collagen was incubated in the standard assay system, it induced the uptake (removal from the soluble phase) of both calcium and phosphate ions. Both the ions were found to be progressively removed from the soluble phase until about 8 h, the time when the limiting velocity was attained. No significant further uptake of either ion took place even when the incubation was further prolonged to 24 h. The studies (Table 1) further revealed that the addition of fluoride significantly

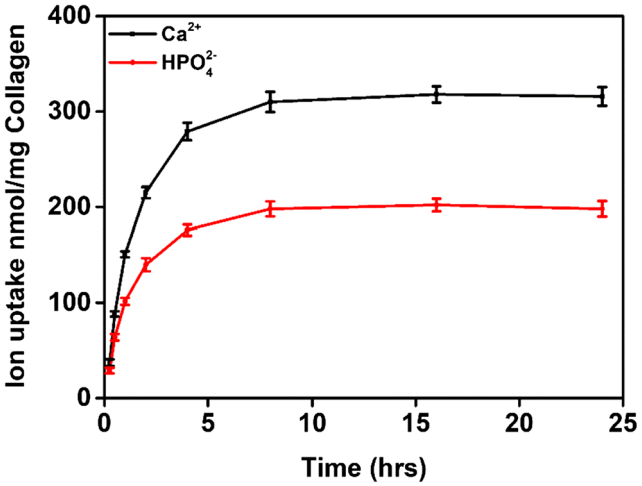

Fig. 1 Collagen-induced in vitro ion uptake (mineralization). One hundred milligrams of collagen was incubated at 37 °C in the assay system consisting of 87.5 mM Tris–HCl buffer (pH 7.4), 105 mM NaCl, 2.04 mM $CaCl_2$, and 1.28 mM KH_2PO_4 in a final volume of 25 ml. The final volume was made by using glass distilled water. Aliquots removed at different time intervals were filtered to determine the concentration of calcium and phosphate ions. Specific ion uptake taking place at a specific time interval was determined by subtracting the concentration of the ion present at that time interval from its concentration present at the start of incubation

influences the uptake of both calcium and phosphate ions from the soluble phase. The addition of fluoride up to a concentration of 5×10^{-7} M was found to have no effect on the disappearance of either ion from the soluble phase. Fluoride at 5×10^{-6}, 1×10^{-5}, 2×10^{-5}, or 5×10^{-5} M concentrations on an average was found to inhibit the uptake of the calcium and phosphate ions by 10, 21, 35, or 54 and 8, 18, 30, or 45% respectively. Interestingly when the fluoride concentration was raised to 1×10^{-4}, 2×10^{-4}, 5×10^{-4}, or 1×10^{-3} M, instead of inhibiting, it was found to stimulate the disappearance of calcium and phosphate ions from the soluble phase on an average by 31, 54, 70, or 98 and 21, 42, 55, or 93% respectively.

Studies on Demineralization:Release of Calcium and Phosphate Ions from the Collagen Bound Mineral Phase into the Soluble Phase and the Effect of Fluoride on It Studies (Table 2) demonstrate that when premineralized collagen preparations having a known amount of calcium and phosphate ions bound as mineral phase were reincubated in the standard reaction system in the absence of calcium and phosphate ions, a significant release of calcium and phosphate from the collagen bound mineral phase into the soluble phase (demineralization) took place. On an average, approximately 10–11% of matrix bound calcium ions and 8–9% of phosphate ions were found to be released in the soluble phase. Percent release of ions was independent of the amount of ions associated with the fixed amount of the matrix. Maximum release of ions was attained at 30 min of incubation and no further demineralization took place even when the incubation was prolonged to 24 h.

Results (Table 3) further revealed that in the presence of 1×10^{-4}, 2×10^{-4}, 5×10^{-4}, or 1×10^{-3} M of fluoride on an average inhibited the release of calcium and phosphate ions from the collagen bound mineral phase by 34, 48, 76, or 94 and 28, 42, 68, or 90% respectively.

Table 1 Effect of fluoride on the rates of ion uptake induced by collagen

Exp.	Concentration of fluoride used (M)	Percent change in the rate of ion uptake (nmoles/mg collagen/15 min)			
		Inhibition		Stimulation	
		Ca^{2+}	HPO_4^{2-}	Ca^{2+}	HPO_4^{2-}
1	5×10^{-9}	-	-	-	-
2	5×10^{-7}	-	-	-	-
3	5×10^{-6}	10.0 ± 2.5	8.2 ± 1.9	-	-
4	1×10^{-5}	21.4 ± 3.7	18.4 ± 2.6	-	-
5	2×10^{-5}	35.2 ± 3.3	30.2 ± 4.1	-	-
6	5×10^{-5}	54.0 ± 6.6	45.0 ± 5.9	-	-
7	1×10^{-4}	-	-	31.2 ± 2.8	21.3 ± 1.9
8	2×10^{-4}	-	-	54.1 ± 4.1	42.4 ± 3.5
9	5×10^{-4}	-	-	69.8 ± 3.2	55.0 ± 4.6
10	1×10^{-3}	-	-	98.0 ± 1.8	92.6 ± 5.6

All values are mean ± S.D of five replicates

Assay system, given under Fig. 1, was employed to determine the effect of fluoride on the rates of ion uptake induced by collagen. Suitable amounts of fluoride were added in the reaction system to give its specified concentrations. Percent inhibition or stimulation caused by a specific amount of fluoride was calculated with respect to the control system where identical amounts of glass distilled water were added

Table 2 Release of calcium and phosphate ions (demineralization/dissolution) from the collagen bound mineral phase

Exp.	Ions present in the mineral phase bound to collagen (nmoles/mg collagen)		Release of ions from the mineral phase bound to collagen (nmoles/mg collagen)		Percent release of the ions from the mineral phase bound to collagen	
	Ca^{2+}	HPO_4^{2-}	Ca^{2+}	HPO_4^{2-}	Ca^{2+}	HPO_4^{2-}
1	320.0 ± 14.6	210.3 ± 10.6	38.2 ± 3.6	18.1 ± 2.6	11.8 ± 3.2	8.6 ± 2.5
2	480.2 ± 12.8	320.6 ± 14.5	54.7 ± 6.5	28.6 ± 1.9	11.2 ± 4.1*	8.9 ± 1.9*
3	680.3 ± 21.2	481.2 ± 14.1	78.1 ± 4.7	42.2 ± 3.2	11.4 ± 3.6*#	8.8 ± 2.0*#

All values are mean ± S.D of five replicates. *NS from Exp. 1 ($p < 0.001$) and # NS from Exp. 2 ($p < 0.001$)

Different amounts of calcium and phosphate solutions were employed in the reaction system given under Fig. 1, to obtain increasing amounts of ions present in the mineral phase bound to collagen. After 24 h of incubation, the mineralized collagen samples thus obtained were washed twice with Tris–HCL buffer to remove the adventitiously bound ions. Calcium and phosphate ions present in the mineral phase bound to the collagen were determined by releasing the bound ions by incubating each representative mineralized collagen preparation in 25 ml of 0.1 N HCL for 15 min. Calcium and phosphate concentrations in the media were determined by the standard methods. The premineralized collagen preparations having different amounts of bound ions thus obtained were then reincubated in the reaction system given under Fig. 1, without the presence of $CaCl_2$ and KH_2PO_4. The amounts of calcium and phosphate ions thus released represented the magnitude of demineralization. Maximum release of ions was attained at 30 min of incubation and no further demineralization took place even when the incubation was prolonged to 24 h

Effect of Magnesium and Pyrophosphate on the Ability of Fluoride to Influence the Mineralization and Demineralization Reactions Studies (Table 4) showed that 1×10^{-4} M magnesium was found to inhibit the uptake of calcium and phosphate ions by approximately 37 and 21% respectively without having any effect upon the release of ions, i.e., demineralization. In contrast to magnesium, at 1×10^{-5} M concentration, pyrophosphate was found to significantly inhibit both the rates of ion uptake and extend of ions release. As observed previously, fluoride at 1×10^{-5} M concentration was found to significantly inhibit and at 2×10^{-4} M concentration stimulate the collagen-induced uptake of both calcium and phosphate ions. The addition of 1×10^{-4} M magnesium along with 1×10^{-5} M fluoride was found to have a highly significant additive effect on their abilities to independently inhibit the uptake of both calcium and phosphate ions without having an effect upon demineralization. When 2×10^{-4} M fluoride was added along with 1×10^{-4} M magnesium, although in the presence of 1×10^{-4} M magnesium, the ability of fluoride to stimulate collagen-induced ion uptake decreased highly significantly by approximately 50%, yet, magnesium was found to have no impact on the ability of fluoride to influence the release of ions. The above studies further revealed that although at 1×10^{-5} M concentration of fluoride was found to enhance the inhibitory effect of 1×10^{-5} M pyrophosphate on collagen-induced uptake of ions, yet, it was found to have no significant impact on the ability of pyrophosphate to influence the release of ions. However, at 2×10^{-4} M concentration, fluoride was not only found to highly significantly decrease

Table 3 Effect of fluoride on the release of calcium and phosphate ions from the collagen bound mineral phase into the soluble phase (demineralization)

Exp.	Concentration of the fluoride used (M)	Release of ions from the collagen bound mineral phase (nmoles/mg collagen)		Percent inhibition of the release of collagen bound mineral phase ions	
		Ca^{2+}	HPO_4^{2-}	Ca^{2+}	HPO_4^{2-}
1	Control	36.0 ± 3.4	18.2 ± 2.0	-	-
2	1×10^{-4}	23.8 ± 2.6	13.1 ± 1.8	34 ± 3*	28 ± 2*
3	2×10^{-4}	18.7 ± 1.3	10.6 ± 1.4	48 ± 2*#	42 ± 4*#
4	5×10^{-4}	8.6 ± 0.9	5.8 ± 0.8	76 ± 4*#@	68 ± 3*#@
5	1×10^{-3}	2.2 ± 0.6	1.8 ± 0.7	94 ± 5*#@$	90 ± 7*#@$

All values are mean ± S.D of five replicates. *Highly significant from Exp. 1 ($p < 0.001$); # highly significant from Exp. 2 ($p < 0.001$); @ highly significant from Exp. 2 and 3 ($p < 0.001$); and $ highly significant from Exp. 2, 3, and 4 ($p < 0.001$)

Different amounts of fluoride were added to the demineralization assay system given under Table 2 to determine its effect on demineralization. Percent inhibition caused by a specific amount of fluoride was calculated with respect to the control system where identical amounts of glass distilled water were added

Table 4 Effect of magnesium and pyrophosphate in the absence and presence of fluoride on collagen-induced mineralization and demineralization reactions

Exp.	Reaction system control plus	Percent inhibition*/stimulation** of the rates of collagen-induced ion uptake (mineralization).		Percent Inhibition of demineralization (ions release)	
		Ca^{2+}	HPO_4^{2-}	Ca^{2+}	HPO_4^{2-}
1	1×10^{-4} M Mg^{2+}	36.8 ± 3.9*	21.1 ± 3.1*	-	-
2	1×10^{-5} M PPi^{4-}	43.6 ± 1.2*	42.6 ± 0.7*	34 ± 4	32 ± 3
3	1×10^{-5} M F^{1-}	23.4 ± 2.7*	17.2 ± 2.4*	-	-
4	2×10^{-4} M F^{1-}	53.6 ± 2.8**	43.2 ± 2.2**	37 ± 3	31 ± 4
5	1×10^{-4} M Mg^{2+} and 1×10^{-5} M F^{1-}	52.2 ± 2.4*$^{@}$	42.8 ± 2.4*$^{@}$	-	-
6	1×10^{-4} M Mg^{2+} and 2×10^{-4} M F^{1-}	25.2 ± 2.8**$^{\#}$	21.0 ± 2.0**$^{\#}$	36 ± 4$^{\alpha}$	33 ± 2$^{\alpha}$
7	1×10^{-5} M PPi^{4-} and 1×10^{-5} M F^{-}	62.2 ± 1.4*$^{\$}$	59.9 ± 0.9*$^{\$}$	35 ± 5$^{\beta}$	31 ± 6$^{\beta}$
8	1×10^{-5} M PPi^{4-} and 2×10^{-4} M F^{-}	18.2 ± 1.4*$^{\%}$	15.9 ± 0.8*$^{\%}$	69 ± 5$^{\lambda}$	61 ± 4$^{\lambda}$

All values are mean ± S.D of five replicates. $^{@}$ Highly significant from Exp. 1 and 3 ($p < 0.001$); $^{\#}$ highly significant from Exp. 1 ($p < 0.01$) and from Exp. 4 ($p < 0.001$); $^{\$}$ highly significant from Exp. 2 and 3 ($p < 0.001$); $^{\%}$ highly significant from Exp. 2 and 4 ($p < 0.001$); $^{\alpha}$ NS from Exp. 4 ($p < 0.001$); $^{\beta}$ NS from Exp. 2 ($p < 0.001$); and $^{\lambda}$ highly significant from Exp. 2 and 4 ($p < 0.001$)

Independent mineralization and demineralization studies were conducted as per the assay systems given under Tables 1 and 3 respectively. Percent inhibition or stimulation of reactions was calculated with respect to the respective control systems where identical amounts of glass distilled water were added

the ability of 1×10^{-5} M pyrophosphate to inhibit the collagen-induced uptake of ions but also enhance its ability to inhibit the release of ions.

Discussion

Studies (Fig. 1) demonstrate that collagen catalyzes the uptake of calcium and phosphate ions from the stable solutions to form matrix bound mineral phase. The studies further revealed that with the increase in the time of incubation, the ratio at which calcium and phosphate ions disappear from the media to form matrix bound mineral phase gets progressively increased. When the limiting velocity was attained, the ratio at which calcium and phosphate ions disappeared from the media closely resembled to the ratio of 1.67:1 present in hydroxyapatite. It thus clearly suggests that the mineral phase formed under the present experimental conditions resembles hydroxyapatite in nature. The studies further revealed that the calcium and phosphate ions removed from the soluble phase get tightly associated with the matrix to form the mineral phase and the ions of the mineral phase thus formed cannot be removed from their matrix association by either washing with glass distilled water or buffer. An almost 100% release of the matrix bound ions was obtained by incubating the mineralized matrices with 0.1 N HCl for 10 min. The above observations are in conformity with our earlier studies and the findings of various other workers [1, 7, 8].

The studies (Table 1) revealed that at lower concentrations fluoride significantly inhibited while at higher concentrations it significantly stimulated the rate of mineralization. Maximum inhibition of approximately 50% was attained by a fluoride concentration of 5×10^{-5} M while an almost 100% stimulation occurred at a fluoride concentration of 1×10^{-3} M. Studies (Table 2) further revealed that when the pre-mineralized collagen preparations were resuspended in the reaction media in the absence of calcium and phosphate ions, demineralization (release of ions from the matrix bound mineral phase into the soluble phase) takes place. A maximum of approximately 10% of the bound ions were found to be released and the percent release of ions was found to be independent of the amount of the ions present in the matrix bound mineral phase (NS effect). The above observation can be explained on the basis that demineralization/dissolution of the mineral phase must stop when the ion product of the released ions becomes equal to the solubility product of the mineral phase. In addition to influencing the rate of mineralization, fluoride at higher concentrations was also found to highly significantly inhibit the extent of demineralization (Table 3). Increasing the fluoride concentration from 1×10^{-4} to 1×10^{-3} M, was found to increase its ability to inhibit the extent of demineralization from 30 to almost 100%. The duel effect of fluoride on mineralization is well known as fluoride is not only used in toothpastes to prevent dental caries but it is also known to cause skeletal fluorosis [19–22].

The concentration dependent effect of fluoride to influence the rate of mineralization and the extend of demineralization was further investigated in the presence of magnesium and pyrophosphate, the two well-known inhibitors of mineralization and/or demineralization, postulated to be involved in the control of biological mineralization. Mg^{2+} has been shown to inhibit mineralization by blocking an obligatory binding of

Ca^{2+} to a specific site on the matrix involved in initiating the process of mineralization, without having any effect on demineralization. On the other hand similar to the action of crystal poisons, pyrophosphate has been shown to inhibit both the mineralization and the demineralization reactions by binding to the mineral phase [7, 8]. Results (Table 4) demonstrate that magnesium and fluoride at lower concentrations ($< 1 \times 10^{-4}$ M) may be acting by the same mechanism to influence collagen-induced in vitro mineralization. The effects of lower concentrations of fluoride and magnesium to inhibit mineralization were found to be additive in nature. The stimulation caused by higher concentrations ($> 1 \times 10^{-4}$ M) of fluoride was found to be significantly reduced in the presence of magnesium. However, the inhibition of demineralization, caused by higher concentrations of fluoride, was not influenced by magnesium.

Similar to the studies on magnesium, the inhibitory effects of both pyrophosphate and lower concentrations fluoride on mineralization were found to be additive in nature. The stimulation of mineralization caused by higher concentration of fluoride ($> 1 \times 10^{-4}$ M) was converted to inhibition in the presence of pyrophosphate. In contrast to the effect of magnesium, when pyrophosphate and high concentration of fluoride were added together, their effect on demineralization was found to be additive in nature. The above results can be interpreted to mean that at its lower concentrations the effect of fluoride on mineralization and demineralization is similar to that of magnesium. However, at its higher concentrations, the effect on mineralization is opposite to both of magnesium and pyrophosphate. Similar to the action of crystal poisons [25], both pyrophosphate and fluoride at its higher concentrations were found to inhibit demineralization and their actions were additive in nature.

Fluoridated toothpastes are widely used all over the world to prevent dental caries. There is no minimum fluoride concentration prescribed to be essentially present in the toothpastes. Desirable concentration of fluoride in toothpastes depends upon the age of the person, his country, and the severity of the tooth decay. Concentration of fluoride in toothpastes normally varies between 1000 and 1500 ppm. Oral health professional sometimes recommend to adults having advanced dental caries the use of the toothpaste having 5000 ppm fluoride [26, 27]. Fluoride, at high concentrations when given to the females during gestation, is known to pass through the placental barrier to adversely affect the development of teeth and jaws of neonates [28].

Fluoride is added in the toothpastes to prevent or cure dental caries by acting tropically only, without getting ingested and entering the body. Thus, to have an approximate idea about the concentration of fluoride which significantly influences the process of mineralization or demineralization in the body, it may not be desirable to compare the concentration present in toothpastes with those used either in vivo or in vitro studies to have any significant effect on the mineralization or demineralization reactions in the body. Present studies (Table 1) demonstrate that fluoride at concentrations less than 5×10^{-6} M (5 μM) has no influence on in vitro mineralization or demineralization reactions. It is interesting to note that this concentration corresponds to the concentration of 5–8 μM fluoride present in human plasma. In addition, the observations made during the present studies (i.e., mineralization or demineralization reactions get influenced only at fluoride concentration significantly greater than 5 μM) and the findings of the other researchers that for skeleton fluorosis to occur in human being fluoride concentration has to be much higher than 10 μM and could be as high as 40 μM [29, 30] clearly suggest that present studies have physiological significance.

Further studies are in progress to investigate the possible mechanism(s) by which different concentrations of fluoride act to influence in vitro mineralization and demineralization reactions. It is hoped that once the mechanisms by which low and high concentrations of fluoride act to either prevent caries or cause fluorosis are known, it may help the oral health professional to correctly optimize the total fluoride exposure from all sources to produce the desired beneficial effects.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

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