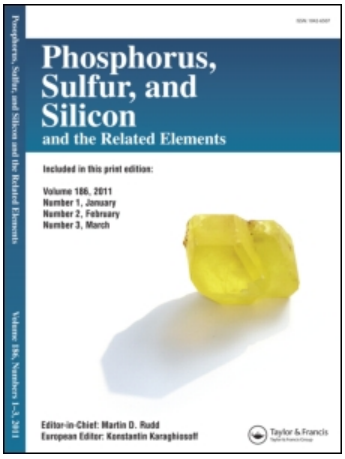

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Effect of In-Vitro Fluoride Ion Treatment on the Mechanical Properties of Bone Tissue

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Cortical bone tissue has been modeled as a short fiber reinforced composite material. It is composed of an organic matrix comprised mainly of Type I collagen reinforced by a mineral crystal similar to carbonated hydroxyapatite. We have developed an in-vitro ion treatment method to alter mechanical properties of the bone tissue using fluoride ion solutions. When bone is exposed to a high ionic strength fluoride solution, part of the bone mineral dissolves and precipitates as calcium fluoride. Changes in the mechanical properties such as, elastic modulus, ultimate stress and ultimate strain are dependent on the concentration and the pH of the treatment. Mechanical properties of fluoride treated (high ionic strength) bone tissue showed that elastic modulus, yield stress and ultimate stress are reduced in comparison to the controls. However ultimate strain increased considerably as compare to controls indicating that there might be a slippage between the bone mineral and the organic phase.

Keywords: Bone tissue; bone mineral; fluoride ions; mechanical properties; composite behavior; bone mineral content (BMC)

Introduction

Cortical bone can be modeled as a short platelet reinforced two-phase composite material. The organic matrix phase is primarily made of type I collagen. The mineral phase is made of a carbonated hydroxyapatite like structure.

The mechanical properties of bone tissue depend on the volume fraction of the mineral platelets, the physical properties of platelets and the mechanical properties of organic phase. The interfacial bonding between the platelets and the organic phase is also a major determinant of the mechanical properties of bone tissue.

In this in-vitro study, the effects of high concentration fluoride ion treatment technique on the tension mechanical properties of bone tissue were investigated. At high ionic fluoride concentration, hydroxyapatite type materials dissolve partially and precipitate mostly as calcium fluoride. Precipitated calcium fluoride can create a protective layer on the hydroxyapatite like surfaces and prevent further dissolution of the

material. By using this technique, the composite behavior of bone tissue is examined in terms of BMC and interfacial bonding.

Materials and Methods

Tension samples were prepared from the medial portion of the diaphysis of bovine femurs. Bones were kept wet during all phases of preparation and during the experiments. Dumbbell shaped samples were prepared parallel to the long bone axis. Demineralized bone tension samples were prepared in order to examine the effects of ion treatments on the organic matrix of bone tissue by using EDTA. The treatment protocol involved an initial 24 hour treatment with a non-ionic detergent solution (0.1 % Nonidet P40 (NP 40)) followed by 72 hour equilibration in ion containing solutions at room temperature or at 37°C. The pH of the solutions was determined. Treatment solutions for control samples contained 0.145M saline at pH=7.5 , 0.145M saline pH=10.5 , 2.0M NaCl pH=7.5 and 2.0M NaCl pH=10.5. For the fluoride treatments 0.145M NaF (Buffered) pH=7.5 , 0.5M NaF (Buffered) pH=7.5 and 2.0M NaF pH=7.5 (With and without buffer) were used. In intact samples; total fluoride, calcium fluoride and densities were measured. In treatment solutions; the calcium contents, the pH changes during the treatments and the total protein amounts were measured. Uniformity of the treatment across the cross section of the bone samples (2mm x 5mm) were determined by either Energy Dispersive X-Ray Spectroscopy or Scanning Acoustic Microscopy. Tensile testing was performed with a gage length of 12.5 mm at a strain rate of 2.65×10^{-3} s^{-1}. The yield stresses, ultimate stresses, elastic modulus and ultimate strains were determined. The demineralized samples were tested by protecting the sample ends with a paraffin wax coating.

Results

Ash weight analysis and wet density measurements showed that all the samples had similar mineral content and wet density. Total protein assays showed that less than 0.017 ± 0.002 % of the available protein dissolved due to any of the treatments. The pH of the sodium fluoride treatment solution (2.0M not buffered) changed during the 72 hours of treatment and its pH's rose from an initial value 7.5 to about 10.1 at the end of the treatment. The amount of calcium in the saline treatment solution was 17.1 ppm in control samples. This indicated that approximately 0.08 % of the bone mineral dissolved in the control samples. The amount of calcium in the fluoride treatment solutions was negligible. The weight of fluoride as a percentage of the mineral weight in control and fluoride treated samples (2.0M Not buffered) was 0.08 ± 0.01 % and 1.76 ± 0.17 %, respectively. The amount of fluoride in the 1M KOH supernatant after 24 hours indicated that 63% of the total fluoride in fluoride treated (Not buffered) bone sample was present as CaF_2. On the same sample, NMR measurement showed that about 89% of the total fluoride was present as CaF_2. Energy dispersive X-ray spectroscopy and Acoustic Scanning Microscopy showed that fluorine content across the bone samples (2.0M

Fluoride treated) was quite uniform. The mechanical properties of bone samples are summarized in Table 1.

Table 1: Mechanical Properties of Intact samples

Groups	Modulus E Gpa.	Yield Stress Mpa	Maximum Strain %
Control Samples	20.42(1.52)	116.8(15.3)	2.86(1.83)
2.0 M Fluoride (Not buffered) at room temp.	16.8 (1.7)	83.0 (13.1)	
0.145 M Fluoride(Buffered) at pH=7.5, 37°C	18.0(1.77)	101.6(13.0)	4.47(1.62)
0.5 M Fluoride (Buffered) at pH=7.5, 37°C	14.47(2.49)	77.5(14.3)	5.35(1.67)
2.0 M Fluoride (Buffered) at pH=7.5, 37°C	14.4(1.27)	77.1(6.22)	7.4(1.2)

Demineralized sample experiments showed that the fluoride treatments did not affect the organic phase.

Discussion

Figure 1 shows the stress-strain curves for control and buffered samples.

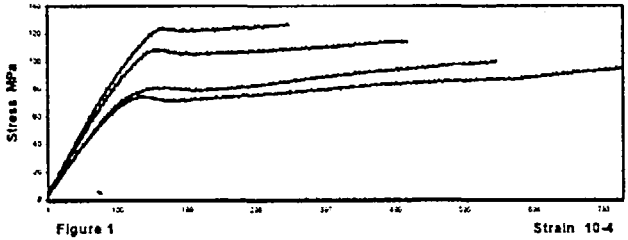

Figure 1

The first curve from the y-axis shows the control sample. Other curves show 0.145M, 0.5M and 2.0M fluoride treated samples (Buffered). Ultimate strain increases with the treatment solution's increasing fluoride content. Two molar fluoride treated samples have the maximum ultimate strain. The increase in the plastic type deformation with increasing fluoride concentration indicates that there is more deformation takes place in the organic phase. This could be due to fewer interactions between the bone mineral and organic phase and/or reduction in the amount of structurally active bone mineral content. Therefore this type of large deformation due to higher fluoride concentration could

indicate that the bonding between the bone mineral and organic phase changes. These results also indicate that the buffered solutions amplify the results as compare to not-buffered treatments (Buffered versus Not buffered 2.0M fluoride treatment)(Table 1). Total fluoride in 2.0M Fluoride treated samples (Not buffered) was about 1.76 % of the mineral weight. In these samples, the amount of fluoride as calcium fluoride was measured 1.57 % (1.76x0.89) by the NMR method. This result showed that about 4.4 % bone mineral was dissolved and precipitated as calcium fluoride. These experiments indicated that the primary mechanism of fluoride action was partial dissolution of the bone mineral and subsequent precipitation as CaF_2. Using an empirical relation between BMC and elastic modulus E (logE = -9.16 + 4.3 x log Calcium, (power law))[1] we can estimate the BMC for the control and the fluoride treated samples (Not buffered). Using the measured values of E in this relation (Table1), we calculated 272 and 260 for the calcium contents (milligram of calcium in 1gm of dry defatted bone tissue) in control and fluoride treated bone samples, respectively. This corresponds with a decrease in the BMC of 4.4 % ((272 - 260)/272). In these calculation, it is assumed that the bone mineral has the following composition ($Ca_{8.35}(PO_4)_{4.35}(HPO_4)_{0.59}(CO_3)_{1.06}(OH)_{0.35}$) [2]. For the 2.0M not buffered fluoride treatment power law predicted the same dissolution value as our experimental prediction. These results showed that by using BMC as an indicator, most of the mechanical properties of bone tissue can be predicted after in-vitro fluoride treatment. Using different ionic strengths, treatment times and pHs, one can obtain bone samples with different mechanical properties. The fluoride treatment affects the mechanical properties of bone tissue by decreasing the structurally effective BMC and altering the interfacial bonding of the mineral-organic interface. This permits us to create mechanically different bone samples from the same bone tissue. This technique allows us to investigate the composite behavior of the bone tissue in an effective way. This study also showed that with the fluoride treatment method, the effect of interfacial bonding on the mechanical properties of bone tissue could be investigated. Our results suggest that the calcium fluoride like precipitate in the bone matrix does not contribute to the mechanical properties of bone tissue in an observable manner.

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