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INVESTIGATION ON THE BIOLOGICAL EFFECT OF FLUORINE PRESENT ON THE SURFACE OF TITANIUM IMPLANTS. ANALYSIS OF THE REACTION FLUORIDE-TITANIUM OXIDE

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Abstract

The aim of this work was to investigate the state of fluoride at the surface of fluoride-modified titanium implants, and to provide information to support or discard the hypothesis that fluoride ion participates in the osseointegration of implants. Titanium is coated with a 2-10 nm thick layer of stable and adherent TiO_2, which reacts with fluoride as a function of the anion concentration. Under present experimental conditions, the half-life of the reaction is about 2 minutes at 17 °C. The stoichiometry of the reaction Ti:F is 0.46 ± 0.03, which do not differs significantly from 0.5, the ratio inferred from the reaction between sodium fluoride and TiO_2: $3\ TiO_2 + 6\ NaF \longrightarrow Na_2TiF_6 + 2\ Na_2TiO_3$. The reaction proceeds as a function of the fraction of anionic fluoride concentration, which increases from a minimum at pH 1 to a maximum close to pH 6. The number of fluoride μmoles that react with TiO_2 grows exponentially as a function of fluoride concentration, towards a maximum at 0.24 M fluoride. According to literature reports, potassium hexafluorotitanate is insoluble in water and it decomposes by heating at 780 °C, indicating a high formation constant. It is concluded that the hexafluorotitanate would not ionize to provide fluoride ion to the implant-bone environment and to promote growth and differentiation of osteoblasts. The effect of fluoride on the surface of titanium sheet was investigated under SEM, showing that the titanium sheet surface is roughened and electron dispersive spectroscopic microanalysis indicated that fluorine is included in the TiO_2 layer.

Key words: fluorine, fluoride, titanium, implants.

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Resumen

INVESTIGACIÓN DEL EFECTO DEL FLUORURO SOBRE LA SUPERFICIE DE IMPLANTES DE TITANIO. ANÁLISIS DE LA REACCIÓN FLUORURO-ÓXIDO DE TITANIO.

El objetivo de este trabajo fue investigar el estado del flúor en la superficie de implantes de titanio tratados con fluoruro de sodio y obtener información para descartar o apoyar la hipótesis de su participación en el proceso de osteo-integración. El titanio está recubierto con una capa estable y adherente de TiO_2, de 2-10 nm de espesor, que reacciona con el fluoruro en función de la concentración del anión. En las condiciones experimentales utilizadas el tiempo de vida media de la reacción es de 2 minutos at 17 °C, pH 5,5. La reacción procede en función de la fracción de fluoruro iónico de la concentración total de fluoruro, que aumenta de un mínimo a pH 1 a un máximo a pH 6. El número de µmoles de fluoruro que reaccionan con TiO_2 crece exponencialmente en función de la concentración del anión, hacia un máximo a 0,244 M. La relación estequiométrica de la reacción Ti:F es 0.46 ± 0.03 no difiere significativamente de 0.5; relación inferida de la reacción: $3\ TiO_2 + 6\ NaF \rightarrow Na_2TiF_6 + 2\ Na_2TiO_3$. Como el hexafluorurotitanato de potasio es insoluble en agua y tiene una elevada constante de formación (se descompone por calentamiento a 780 °C), se concluye que no se disociará para proveer fluoruro a las células óseas en contacto con el implante. La observación al microscopio electrónico de barrido y el análisis por espectroscopía de dispersión de rayos X de la superficie de un trozo de hoja de titanio tratado con solución de NaF a pH 3.5, indicaron aumento de la rugosidad y presencia de flúor en la capa de TiO_2.

Palabras clave: flúor, fluoruro, titanio, implantes.

Introduction

Titanium is a higly biocompatible metal due to its resistance to corrosion, bio-inertness, capacity for osseointegration, and high fatigue limit. Titanium biocompatibility is the result of the protective oxide film that forms naturally in the presence of oxygen. The oxide film is strongly adherent, insoluble, and chemically impermeable, preventing reactions between the metal and its environment.[1]

Treatment of titanium surface with fluoride was one of procedures that initiated the “second generation” of implants. Ellingsen[2] was the first to demonstrate that pretreatment with fluoride improves implant osseointegration. He suggested (but did not investigate) that “the presence of a fluoride coat on the surface of titanium implants stimulates the bone response leading to a connection between titanium and phosphate from tissue fluids. Free fluoride ions will catalyze this reaction and induce the formation of fluoridated hydroxyapatite and fluorapatite in the surrounding bone”. He et al.[3] reported the improved bone response to rough titanium implants treated with hydrofluoric acid/nitric acid (HF/HNO_3) solution. Only two reports on the *in vitro* proliferation of pluripotent mesenchymal cells of different sources or the gene expression of osteoblasts[4,5] employing titanium with fluoride modified surface were published in the last ten years.

Human fluorosis demonstrated that the chronic intake of fluoride has a strong bone anabolic effect. Its effect on the proliferation of osteoblasts has been investigated and two hypotheses (probably complementary) have been published. Lau et al.[6] reported that fluoride ion inhibits an acid protein-tyrosine-phosphatase responsible for the hydrolysis of a phosphate in one or more proteins involved in the MAP-kinase signaling cascade that leads to osteoblasts proliferation. Other investigators (Ammann et al.[7] Caverzasio et al.,[8] Jeschke et al.,[9] Susa M.,[10] Lau et al.[11]) assign the activation of osteoblast proliferation to the synthesis of aluminum fluoride (AlF_4). Aluminum is an oligoelement always present in our circulation. According to these investigators AlF_4 replace the γ phosphate of GTP producing GDP-AlF_4, in the G-protein that acts as agonist regulator of adenylatecyclase. The stability of GDP-AlF_4 maintains the synthesis of cAMP unchecked. The high levels of the latter conduce to osteoblasts proliferation.

The aim of this work was to investigate the state of fluoride at the surface of fluoride-modified titanium implants and to provide information to support or discard the hypothesis that fluoride ion participates in the osseointegration of implants. None of the reports cited above investigated this point.

Materials and Methods

Experimental models: Reaction of titanium oxide with fluoride and reaction of 4% NaF solution, pH 3.5 on the surface of a piece of titanium sheet.

Measurement of fluoride ion concentration: Fluoride was measured by direct potentiometry using an ion selective electrode ORION 94-09 and a reference electrode of Ag/AgCl connected to a digital analogical converter.[12]

Reagents: Titanium oxide.titania (TiO_2, Sigma-Aldrich, ≥ 99% pure) was used suspended in distilled water (20 mg/ml [250 mmol/l]). The suspension was stirred for 30 minutes before using. The TiO_2 suspension was added to the mixture of reagents in aliquots of 100 µl (or smaller when indicated) with a micropipette.

NaF stock solution. A 0.244 M NaF solution was prepared dissolving 10.248 g of the salt (Sigma-Aldrich, purity ≥ 99%) in one liter of distilled water. Standard fluoride solutions were obtained by successive dilutions 1:10 of the stock solution: 0.24 M to 10^{-6} M.

Buffer solutions. Acetic/acetate 1 M buffer pH 5.5. Aliquots of this solution were adjusted with concentrated HCl or NaOH solutions to attain pH 4.5 and 6.5, when indicated.

Glycine/HCl 1M buffer pH 3.5. Aliquots of the solution were adjusted with concentrated HCl to attain pH 3.5, 2.5 and 1.5, when indicated.

Total Ionic Strength Adjustor. To provide constant background ionic strength, aliquots of a 1 M NaCl solution were used as indicate below.

General design of experiments.

Experiments were carried out in triplicate. The table displays the design of the typical experiment. Three 25 ml plastic vials were used for each experiment; buffer, NaCl and standard fluoride solutions were added as indicated. They were placed on a magnetic stirrer with a small stir bar. With the readings of these vials (H column) and the log of the actual NaF concentration (F column) the calibration curve was constructed. The suspension of TiO_2 was added and the mV readings recorded as a function of time (I). The latter allowed the calculation of the fluoride concentration at the end of the reaction with TiO_2 (J), the difference with the actual [NaF concentration (K = F-I) and the number of µmoles consumed in the experiment (L). The suspension of TiO_2 was added either at the beginning or in successive aliquots.

Investigation of the presence of titanium ion (Ti^{+4}) in solution after the reaction between fluoride concentration 0.244 M and TiO_2: The suspension of TiO_2 + fluoride solution was centrifuged at 1000 g for 5 minutes and the presence of Ti^{+4} ion in the supernatant was investigated with chromotropic acid[13] (that

Table 1. Design of a typical experiment. *moles/L, pH=5.5

A	B	C	D	E	F	G	H	I	J	K	L
Vial	**Buffer, ml**	**NaCl 1M, ml**	**NaF std., ml**	**Initial [NaF] std. ***	**Actual [NaF] std.***	**Log [NaF]**	**Initial mV**	**Final mV**	**Final [NaF] ***	**(F$^-$J)= moles/L**	**Δμmolesof F reacting with TiO_2**
1	1.0	1.0	10.0	0.0024	0.00203	-2.692	21.20				
2	1.0	1.0	10.0	0.0244	0.0203	-1.692	17.07				
3	1.0	1.0	10.0	0.2440	0.2030	-0.692	11.87	12.67	0.148	0.055	0.66

gives a red chelate with Ti^{+4} with maximum absorbance at 443 nm) and with sulfuric acid and hydrogen peroxide solutions[14,15] to obtain the peroxo-titanic sulfate (yellow complex, with maximum absorbance at 410 nm).

The Ellingsen´s treatment of fluoride-modified titanium implants:[2] A 1x5 cm piece of titanium sheet (Grade 2, 0.3 mm thick) was immersed in 4% NaF solution in 1 M glycine buffer, pH 3.4-3.6, during 30 minutes. After a brief wash in distilled water the titanium sheet was allowed to dry spontaneously. The latter and a piece of untreated titanium sheet were examined under the scanning electron microscopy and the composition of the oxide layer assessed by energy-dispersive X-ray spectroscopy (EDS).

Statistical analysis. Data is reported as the mean ± standard error. Comparisons were done with ANOVA and covariance analyses. Best fit of linear and nonlinear regressions and their parameters were performed with a computer program (GraphPad Prism 5.0, San Diego, California, USA). The algorithm proceeds by iterating until the difference between two consecutive sums of squares is less than 0.01. The differences were considered significant if the probability of different outcomes is less than 0.05.

Results

The curve of Figure 1 reveals that the addition of 250 μmol TiO_2 to a 0.080M fluoride solution pH 5.5,17°C (vial 3, Table 1) with continuous stirring, reduces the concentration of fluoride at a fast rate. The half time (T½) is 2 minutes and independent of fluoride concentration in the present experimental model.

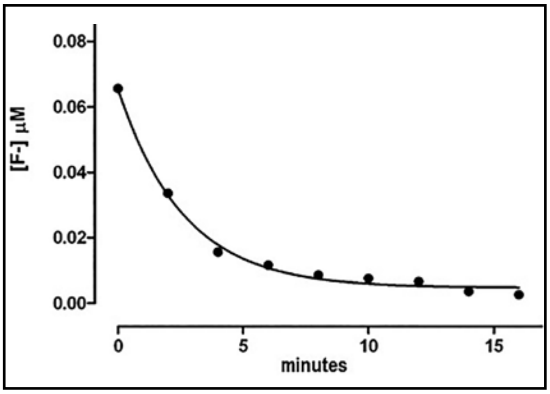

Figure 1. Fluoride concentration as a function of time, after the addition of 250 μmol TiO_2 to a vial containing a 0.080 M fluoride solution, pH 5.5, 17°C.

This experiment was repeated at different pH values and the T½ were compared (Table 2). The analysis of variance of T½ data indicated that they do not differ significantly from each other (p=1.000). It is concluded that the reaction is fast and that this parameter is not affected by acidification of the medium (pH values 4.45 to 6.55).

Table 2. $T_{½}$ of the reaction between 0.1 M fluoride solution and 250 µmol TiO_2 as a function of pH.

pH	$T_{½}$ ± SEM
4.45	1.9±0.3
4.75	2.0±0.3
5.50	2.1±0.1
6.55	2.9±0.5

The measurements of fluoride concentration between pH 1.5 to 6.55 were estimated comparing the parameters of the calibration curves.

Figure 2 shows that the value of the slopes of the calibration curves increased (toward more negative figures) linearly as a function of pH values and the same applies to the values of the ordinates. The analysis of variance reveals that the values of the slopes and the ordinates are significantly affected by pH (Table 3). Difference between slopes: F = 9.267, p<0.0008; differences between ordinates: F = 5.573, p<0.002. As pH values determine the fraction of fluoride in ionic form, these results indicate that the accuracy of fluoride measurements is affected by pH value. It is concluded that the calibration curve of any experiment must have the same pH and ionic strength of the latter.

Table 3. Slopes, ordinates and their SEM of triplicates or calibration curves of fluoride as a function of pH.

pH	Slope	SEM	Ordinate	SEM
1.50	-0.1454	0.00568	1.055	0.112
2.69	-0.1784	0.00478	1.456	0.087
3.68	-0.2098	0.01869	1.823	0.321
4.53	-0.2174	0.00163	1.980	0.028
5.50	-0.2308	0.00615	2.524	0.114
6.30	-0.2134	0.01410	1.875	0.242

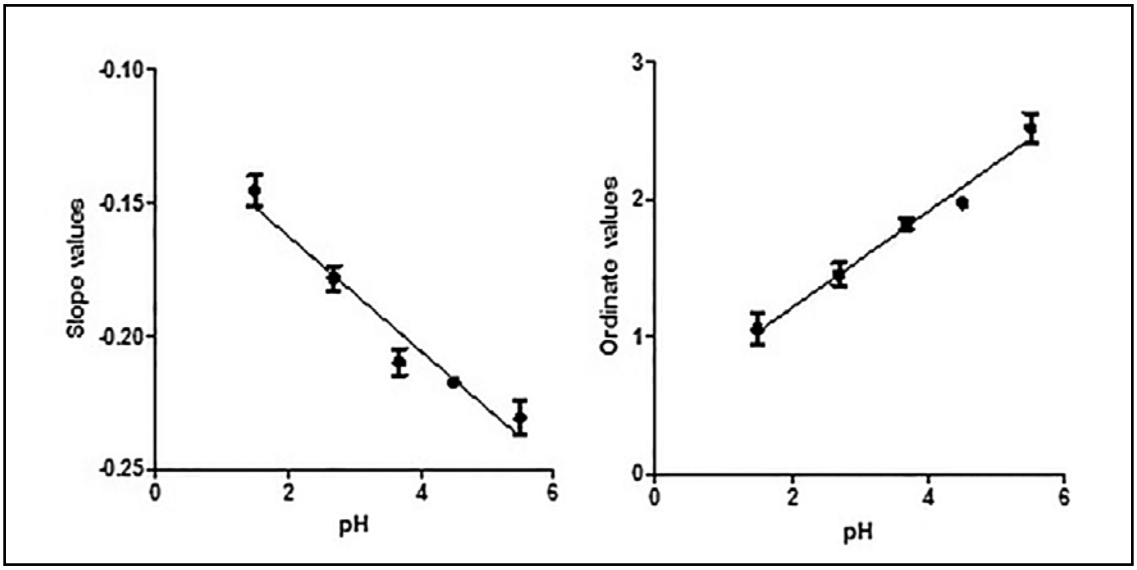

Figure 2. Modification of the slopes (left) and ordinates (right) of fluoride calibration curves as a function of pH values.

Figure 3 exhibits the number of fluoride µmol that react with 250 µmol of TiO_2, (pH 5.5, 17 °C) increases exponentially approaching the fluoride concentration 0.244 M.

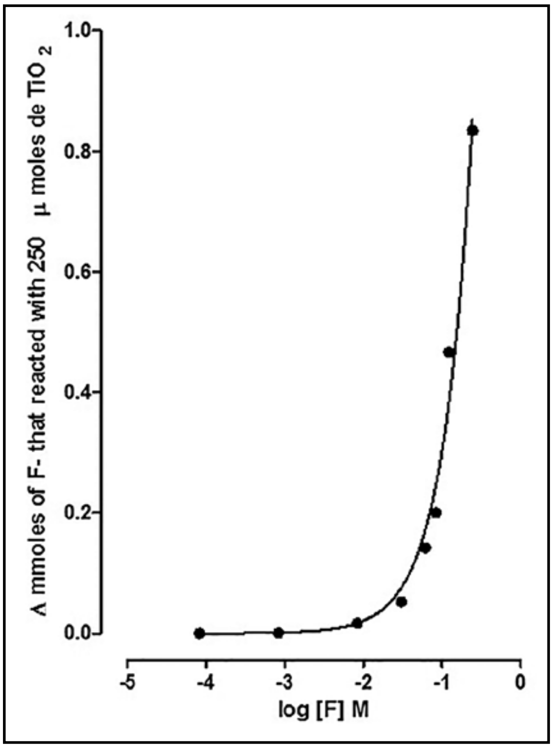

Figure 3. Number of TiO_2 µmol that reacted with fluoride, as a function of the concentration of fluoride solution.

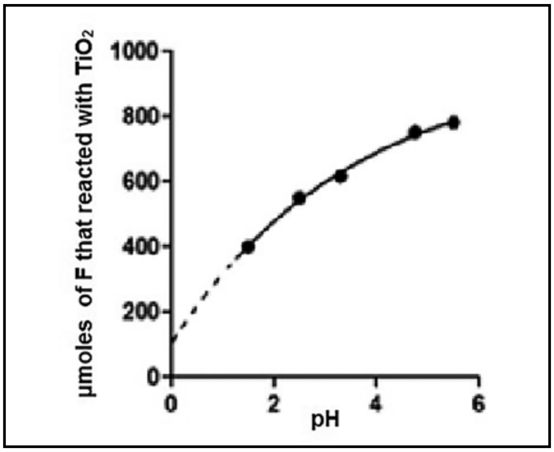

Figure 4. Number of TiO_2 µmol that reacted with fluoride as a function of pH values.

What is the amount of fluoride capable of reacting with 250 µmol TiO_2, as a function of pH?.

The values obtained were adjusted to a growing exponential function (Figure 4) tending to a plateau of the form:

$$Y= Y_o + (Plateau-Y_o)*(1-exp(-K*x))$$

Where Y is the number of fluoride µmol that reacted with TiO_2, Y_o is the ordinate (estimated value = 102 µmol), Plateau (estimated = 964 µmol), K (constant) = 0.2842, R^2 = 0.9920. Clearly the reaction depends on the concentration of ionic of fluoride, which increases approaching neutral pH values.[7]

The following experiment was performed to determine the stoichiometry of the reaction. A series of known amounts of TiO_2 were added successively to a 0.244 M solution of sodium fluoride, pH 5.5, 17 °C. The reaction was left to proceed to completion before each addition of TiO_2 and the number of accumulatedµmoles of fluoride consumed were plotted against the cumulative µmoles of titanium added. The analysis of the results exhibited in Figure 5 reveals a slope: 2.07 ± 0.13 mol fluoride per mol of titanium. This relationship agrees with the ratio F:Ti of the reaction[6] whose products are sodium-hexafluortitanate and sodium titanate.

$$3\ TiO_2 + 6\ NaF \longrightarrow Na_2TiF_6 + 2\ Na_2TiO_3$$

The presence of titanium ion (Ti^{+4}) was investigated in the supernatant of a mixture of fluoride solution with an excess of TiO_2. The reactions with chromotropic acid or with hydrogen peroxide + sulfuric acid were negative. As discussed below, these results are consistent with the experimental conditions (and also probably under physiological ones). The hexafluortitanate complex would not add fluoride ion to the implant environment.

The above information led us to investigate the state the chemical composition at the surface of a polished titanium sheet etched

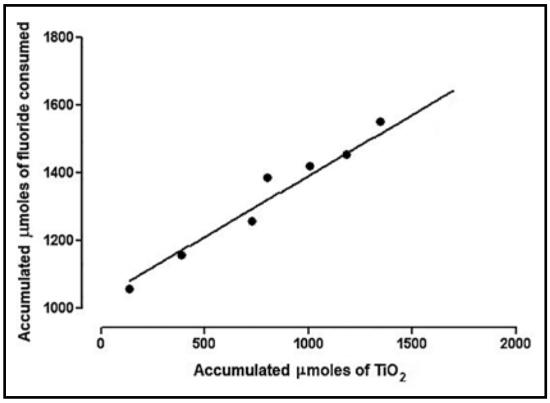

Figure 5. Relationship between the fluoride µmol consumed as a function of the number of µmoles added to the reaction mixture.

Table 4. Microanalsis by EDS of the surface of untreated and F-modified titanium sheet according to Ellingsen.[5]

	Untreated Titanium		F-modified Titanium	
Element	Wt %	At %	Wt %	At %
Carbon	12.81	36.96	2.92	7.29
Oxygen			6.03	9.50
Fluorine			**11.26**	**14.66**
Sodium			1.02	1.08
Chloride			1.66	1.40
Titanium	85.27	61.01	54.62	34.13
Lead			5.87	0.85

with 4% NaF solution pH 3.5, according to Ellingsen.[2] The results obtained were: a) EDS microanalysis indicated the presence of fluorine and b) treatment increased the roughness of the surface (Figure 6, Table 4).

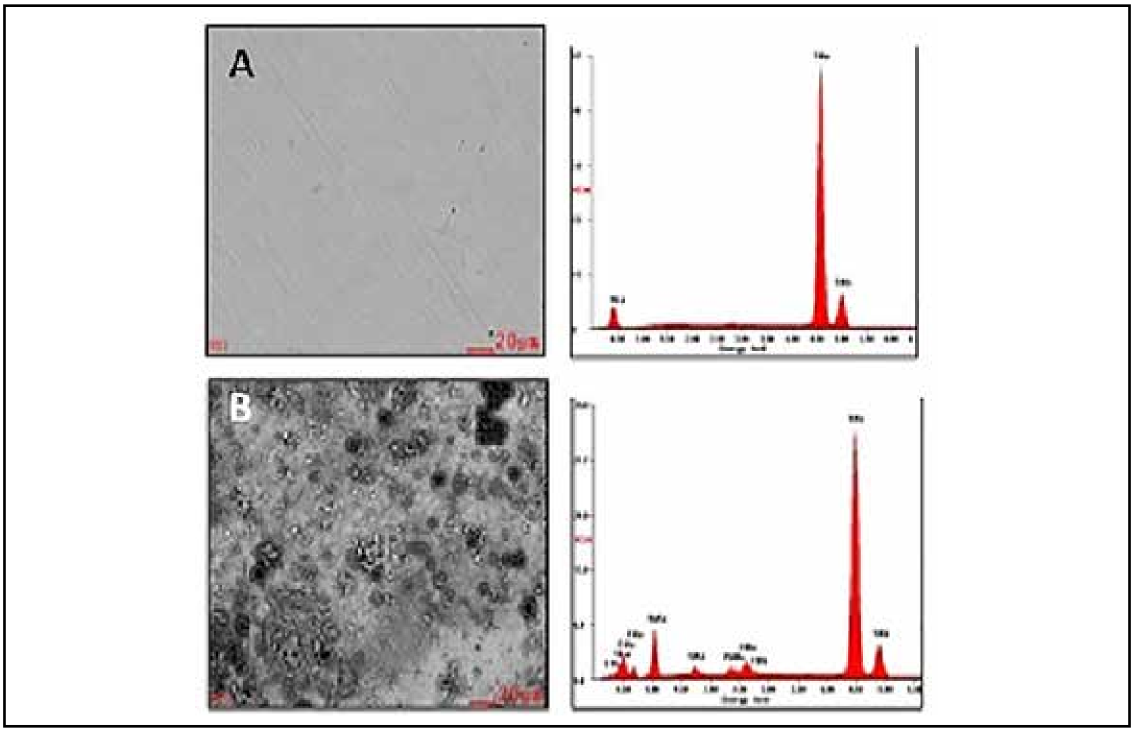

Figure 6. SEM images, spectra of X-ray dispersive energies and elements present in the surface off A:polishedtitaniumsheet and B: F-modified titanium sheet according to Ellingsen.[5]

Discussion

Titanium is coated with a 2-10 nm thick layer of stable and adherent TiO_2, which reacts with fluoride as a function of the anion concentration. Under the stated experimental conditions, the half-life of the reaction is about 2 minutes at 17 °C, pH = 5.5. The stoichiometry of the reaction Ti:F is 0.46 ± 0.03 which does not differ significantly from the theoretical ratio inferred from the reaction between fluoride and TiO_2 giving sodium hexafluorotitanate and sodium titanate as products.[16]

$$3\ TiO_2 + 6\ NaF \longrightarrow Na_2TiF_6 + 2\ Na_2TiO_3$$

The reaction proceeds as a function of anionic fluoride concentration, which increases from a minimum at pH 1 to a maximum close to pH 6.[17] The number of µmoles of fluoride which reacts with TiO_2 grows exponentially as a function of fluoride concentration, toward a maximum at 0.244 M, fluoride.

Potassium hexafluorotitanate is insoluble in water and it decomposes by heating in air over 780 °C[18,19] (giving TiO_2 and KF), indicating a high formation constant. It is concluded that the hexafluorotitanate would not dissociate to provide fluoride ion to the implant-bone environment, to promote growth and differentiation of osteoblasts.

The overall information afforded by present experiments allows us to conclude that a) fluorine can be included in the TiO_2 layer, most probably as insoluble hexafluorotitanate and b) treatment with fluoride roughened the surface of polished titanium, in agreement with the report by Korotin et al: “acid treatment of Ti reduces the content of hydrocarbons increasing the surface energy and bio-compatibility. In addition oxygen concentration on the surface of F-modified Ti is much higher than for the untreated sample, because the acid treatment removes contaminants, increases their reactivity, and provides a better passivation and formation of thick protecting TiO_2 layer”.[20]

It is concluded that fluoride reacts with the titanium dioxide that covers the surface of the metal producing hexafluorotitanate, a complex and highly stable anion that, under physiological conditions would not dissociate to provide fluoride anions in the vicinity of the implants.

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