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Mechanical Assessment of Oseointegration of Implant Coating with Mixture of Strontium Chloride and Hydroxyapatite at Different Concentration

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ABSTRACT

Implant is the future restoration the lost teeth missing and tissues that depend on the stability of the implant in the bone is important to shorten patient wait troublesome period and accelerate the restoration of function and aesthetics.The aim of the study was to evaluate the bond strength between the bone and Ti implant coated with mixture of $Srcl_2$ and hydroxyapatite after different periods of implantation (2, 6) weeks in rabbit tibia by torque removal test.36 screw shaped commerically pure titanium implants, 3.0 mm in diameter and 8mm in length coating with mixture of strontium chloride and hydroxyapatite at different concentration by electrophortic deposition , 18 screws were coated with mix 1 (75%Sr-25%HA) and other 18 were coated by mix 2 (25%Sr-75%HA) . The assess surface topography was done by using optical microscope and phase analysis by X-ray Diffractometer . The screws were implanted in nine healthy adult new zeeland rabbits each tibia received two screws one coated with mix 1 and other coated by mix 2. Torque removal test was done by torque-meter to determine the peak torque necessary to remove the implant from tibia bone after different periods (2 and 6 weeks) of implantation .Torque removal value for mix 2 (25%Sr-75%HA) was statistically significantly higher than mix 1(75% Sr- 25% HA) after 2weeks and non-significant difference after 6 weeks. There was significant increase in torque removal value with time.High torque removal value was obtained by coating titanium screw with mixture 2 (25%Sr-75%HA) than mixture 1 (75% Sr-25% HA).

Keyword: *Torque Removal Test, Coating, RXD, Strontium*

1. INTRODUCTION

The long-term success of dental implants largely depends on rapid healing with safe integration into the jaw bone. Geometry and surface topography are crucial for the short and long-term success of dental implants[1-4] . Implant surfaces have been developed in the last decade in a concentrated effort to provide bone in a faster and improved osseointegration process.[5]

"Osseointegration, defined as a direct structural and functional connection between ordered , living bone and the surface of a load-carrying implant ", is critical for implant stability, and is considered a prerequisite for implant loading and long-term clinical success of endosseous dental implants. [6]

A torque wrench test has been used to quantitatively assess clinical osseointegration of dental implants. If a preload corresponding to a torque of 10 to 20 Ncm is applied to an implant and implant remains stable, without discomfort, the implant can be considered to be osseointegrated.[7]

Strontium (Sr) is a natural bone-seeking trace element that accumulates in the skeleton because of its chemical and physical similarity to calcium and subjected of extensive study over the past several years. [8] The effect of strontium has been shown affecting both bone resorption and bone formation.[9] This effect influences cells activity by acting directly on osteoclasts to inhibit bone resorption [10-12]. The presence of this trace element on the surface of bone at a level higher than that required for normal cell physiology, can be effective in blocking osteoclastic bone resorption without apparent cytotoxic effect on osteoblas. [11] in addition to in vitro study the in vivo studies in different animal models show positive effects of strontium on bone metabolism [12-17]

This study was designed to evaluate the bond strength between the bone and Ti implant coated with mixture of $Srcl_2$ and hydroxyapatite after different periods of implantation (2, 6) weeks in rabbit tibia by torque removal test.

2. EXPERIMENTAL WORK

Rod -shaped commerically pure titanium grade 2 (supplied by GRS TITANIUM ING.1550 spruse street wooster, ohio 44691 USA), with 6mm diameter, were used in this study. 36 screw shaped implants, 3.0 mm in diameter and 8mm in length (threaded part is 5mm and smooth part is 3mm) and pitch height is 1mm, were machined from cpTi rods using Lathe machine. Before coating preparation, all implants were washed in acetone, alcohol and de-ionized water in an ultrasonic bath, and then dried at 45C. [18]

Electrophoretic deposition (EPD) of SR HA mixture coating were applied by two different concentration. First electrolyte solution was prepared by adding 75% strontium chloride with 25% hydroxyapatite to liter of ethanol with 3.6g Polyvinyl butyral (PVB) , and second electrolyte solution was prepared by adding 75% hydroxyapatite with 25% strontium chloride to liter of ethanol with 3.6g Polyvinyl butyral (PVB).

Different durations and different voltages for coating by Electrophoretic deposition (EPD) were used to select the best suitable parameter for EPD process. Sintering of the coated specimens was carried out for densification using Carbolite furnace. The treatment is done under inert gas (argon), to prevent oxidation of the specimen [19]

The phase analysis of the powders was done by XRD (using 3121 powders X-ray Diffractometer using Cu Ka radiation The 2 θ angles were swept from 20- 60° in step of one degree).the data were analyzed with IBM SPSS software (ver 20, SPSS Inc, IL,USA) using descriptive statistics and T test for each time . Differences were considered statistically significant at ***p***-values < 0.05

The surface topography of the coating was made by using optical microscope (Nikon Type 120, Japan optical microscope) to evaluate the appearance of the coated surface layer of the specimen. The microscope was provided with digital camera type DXM 1200 F. The micrographs were analyzed through Nikon ACT -version 2.62, 2000 software.

Nine healthy adult New Zeeland rabbits weighing 2 -2.5 kg were used. Antibiotic cover with oxytetracycline intramuscular injection was given to exclude any infection.Rabbit were left for two weeks in the same environment before surgical operation**.**

The surgical equipments were sterilized. Sterile gloves,gowns and surgical masks were used. The rabbits were anesthetized by (intramuscular injection of ketamine hydrochloride (1ml/kg Body weight) and xylocaine 2% (1ml/kg B.W).

Both legs were shaved and cleaned with chlorhexidine alcohol. The rabbit was placed in a sterile surgical table. Longitudinal incision was made along the medial aspect of the rabbit right tibia. The periosteum was reflected dorsally to the physis. A hole was made, aproximately 3 mm from the proximal physis with straight hand piece (strong 90,Korea) with a round guide drill of 2.0mm in diameter. The screw was inserted and the same procedure was repeated to the other leg, where another screw was inserted.

Suturing of muscles was done with absorbable catgut suture followed by skin suturing with silk suture The operation site was sprayed with local antibiotic (oxytetracycline spray),then long acting systemic antibiotic (oxytetracycline 0.5ml/kg B.W.) **.** Postoperative care was performed by giving oxytetracycline antibiotic (local and systemic) for 3 days after surgery

After the end of implantation period , the rabbits were anesthesized and incision was made to expose the implant to measure torque removal value by torque-meter.

3. RESULTS

Phase analysis was studied using 3121 powders X-ray Diffractometer using Cu K radiation. The 2ϴ angles were swept from 20- 60° in step of one degree. The peak indexing was carried out based on the JCPDS (joint committee on powder diffraction standards) International Centre for Diffraction Data, ICDD file # 44-1294 for titanium,# 11-0218 for Ti_2O , #25-0891 for $Srcl_2.2H_2O$,#33-1322 for $Srcl_2.H_2O$, # 44-0368 for Ca_4P_2O , # 09-0169 for $Ca_3(PO4)_2$, # 9-432 for HA and all the calcium phosphate peaks reported. (fig 1,2) show the typical XRD patterns of the Sr-HA coating on commerically pure titanium implants.

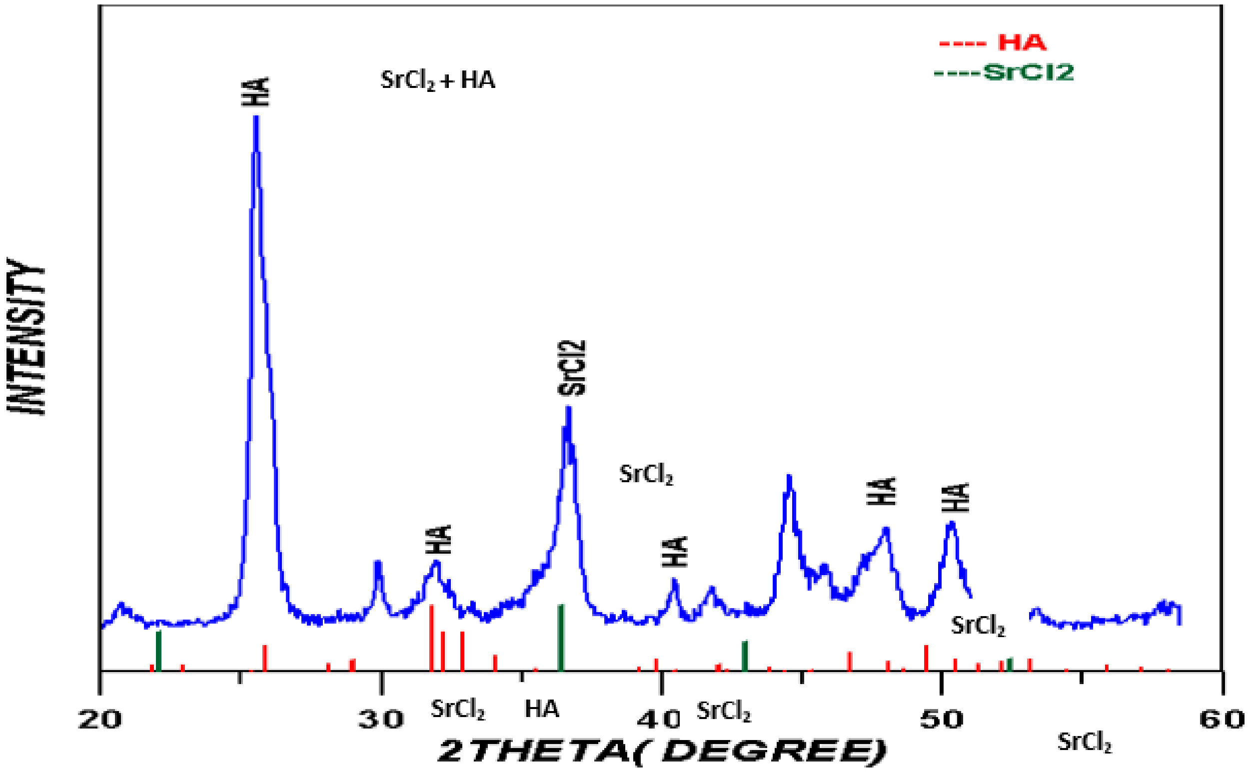

Fig 1 show the typical XRD patterns of the mix 1 (75%Sr-25%HA) coating on commerically pure titanium implants

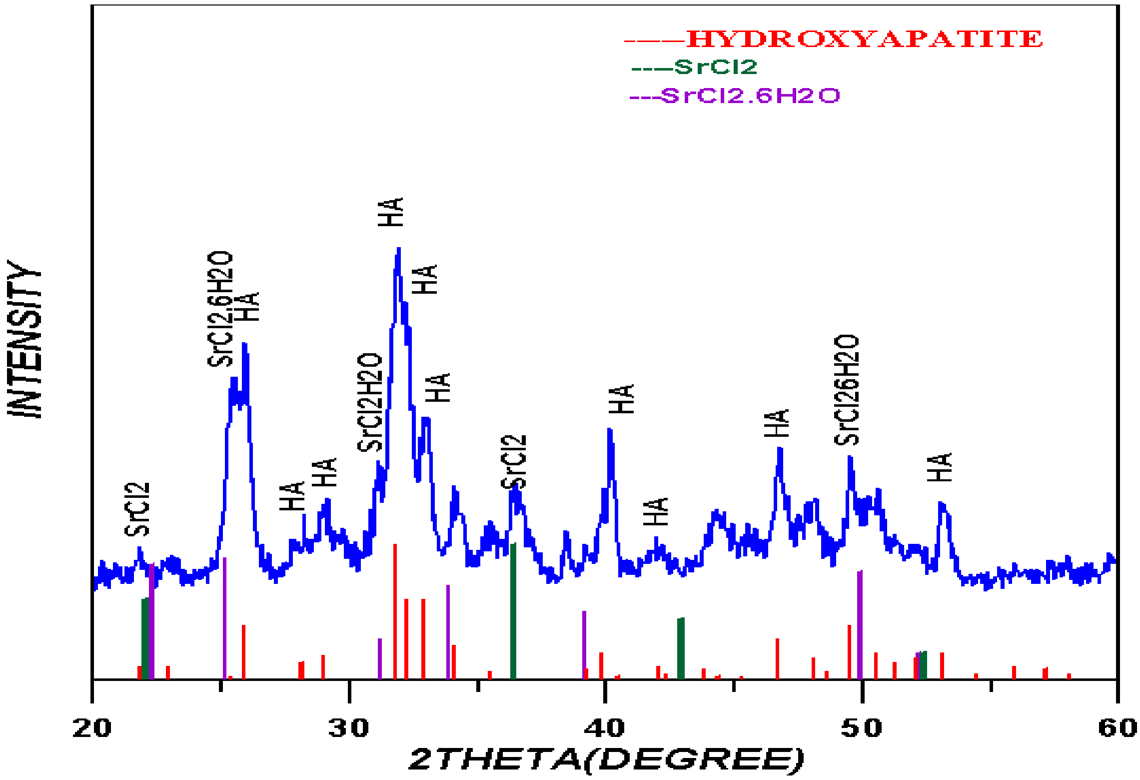

Fig 2 show the typical XRD patterns of the Mix 2 (25%Sr-75%HA) coating on commerically pure titanium implants

Table 1: Comparison of Mean of Torque Removal Value for Mix 1 (75%Sr-25%HA) and Mix 2 (25%Sr-75%HA) Coated Group between 2 and 6 Weeks Intervals

Descriptive Statistics							
time	groups	N	Minimum	Maximum	mean	S. D	S E
2 weeks	Mix 1	9	10.00	22.00	18.055	3.32	1.10
	Mix 2	9	15.00	35.00	25.555	6.24	2.08
6 weeks	Mix 1	9	35.00	75.00	48.666	11.69	3.89
	Mix 2	9	35.00	75.00	54.666	13.26	4.42

Table 2: t –test for equality of means of torque removal value for mix 1 (75%Sr-25%HA) and mix 2 (25%Sr-75%HA) coated implants at 2 and 6 weeks intervals

time	types	T test for equality of means		
		t	df	Sig. (2-tailed)
2 weeks	Mix 1 x Mix 2	3.180	16	.006
6 weeks	Mix 1 x Mix 2	1.018	16	.324

Table 4: t –test for equality of means of NBFR for mix 1 (75%Sr-25%HA) and mix 2 (25%Sr-75%HA) coated implants at 2 and 6 weeks intervals

types	time	T test for equality of means			
		t	df	P value	Sig.
Mix 1	2 weeks x 6weeks	6.456	8	0.000	HS
Mix2	2 weeks x 6weeks	5.095	8	0.001	HS

4. DISCUSSION

The X ray diffraction pattern of coated layer of both mixing ratios of strontium chloride and hydroxyapatite showed that the strontium and hydroxyapatite are existed. XRD showed that narrow peaks were indicative of layer consisted of highly crystalline form , whereas broad peaks represented lower level of crystallinity .[20] Also it can be seen that the amount of $srcl_2$ of both mixes are almost similar inspite of difference in concentration of strontium in the mixture .

The results of the mechanical testing show increase in retention of the implants in the bone in all groups at different periods of implantation. As it is known that strontium has dual effect in stimulating osteoblast formation and blocking osteoclast activation and thus reduce resorption[21,22]. The effect of HA in enhancing bone formation can be seen in different previous researchs[23,24] , thus mixing HA and Sr may lead to increase bone formation due to the effect of both materials.

After 2 weeks , implants coated with Mix 2 (25%Sr-75%HA) recorded significant increase in removal torque value than mix 1 (75%Sr-25%HA) ,this indicated increased bond strength at bone implant interface which may be due to mechanism of action of strontium which depends on ion release and Sr^{+2} becomes absorbed via ion exchange processes with Ca^{+2}, or binds to osteoid proteins, or the incorporation of Sr2+ ions into the crystal lattice of the bone mineral. This indicates that the mechanism of action is a relatively rapid uptake into new bone and longer term exchange process in older bone, Mixing HA-Sr in mix 1 might restricted the release of Sr ion in which mechanism of action depend on this release . X ray diffraction pattern of mix 2 show water molecules which may increase dissolution and release of Sr in mix 2 than mix 1, therefore more Sr ions run to tissue and showed higher torque removal value at 2 weeks. Electrophoretic deposition depend on the particle size and density [25] . The density of Sr is three times greater than that of HA. Also there is difference in particle size between than them which may effect electrophoretic deposition process and difference in coating for two mixes and more power might be needed to move Sr ions than HA ions.

Another explanation of higher torque value for mix 2 (25%Sr-75%HA) than mix 1 (75%Sr-25%HA) may be due to higher concentration of Sr in the solution of mix 1 than mix 2. This might lead to thicker coating layer of mix 1 and because of the too high density of strontium this may lead to stripping of the coating layer and loosing much of strontium ions, while in second mix (25%Sr-75%HA) the amount of strontium was less, so the deposited layer was thinner which help to be bonded to the surface and not stripping off. Thus reasonable amount of strontium left on Ti screw.

There non-significant difference between mix 1 and mix 2 after 6 weeks which may be due to the strontium is dose dependent so with time more strontium released from coating layer , so Sr effect was reduced and the result is more depended on HA action .

Both coating materials showed increased torque removal force after 6 weeks of implantation which is normal physiological process, **Robert et al, 1984** stated that "after insertion of an implant, a poorly organized woven bone with low strength is formed at the interface then maturation of woven bone to lamellar one with adequate strength take about 6 weeks" [26]**.**

4. CONCLUSION

High torque removal value was obtained by coating titanium screw with mixture 2 (**25%Sr-75%HA**) than mixture 1 (**75% Sr- 25% HA**).

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