Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2018, 7(1): 152-157

Histomorphometric Assessment of Implant Coating with A Mixture of Strontium Chloride and Hydroxyapatite at Different Concentration

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ABSTRACT

Background/purpose: *Surface properties are one of the major keys of successful implant osseointegration in addition to mechanical strength and excellent biocompatibility of implant material. The purpose of this study is to make histological and histomorphometric analysis of an implant coated with strontium chloride ($SrCl_2$) mixed with hydroxyapatite (HA) at different concentrations, in rabbit tibia at 2 and 6 weeks of implantation time.* ***Method:*** *48 commercially pure titanium screw shaped implants were placed in 24 healthy adult New Zeeland rabbits, each rabbit received 2 implants; one coated with mixture 1 (25% HA and 75% $SrCl_2$) and the other coated with mixture 2 (75% HA and 25% $SrCl_2$). Twelve rabbits were sacrificed at 2 weeks of healing and other twelve after 6 weeks. The new bone area and number of cells (osteoblast and osteoclast) were assessed by light microscope.* ***Result:*** *Statistical analysis showed significant differences in new bone formation ratio after 2 weeks of healing and non-significant differences after 6 weeks of healing. Data also suggested that osteoblast was increased, and osteoclast was decreased in mixture 2 (75% HA and 25% $SrCl_2$) more than mixture 1 (25% HA and 75% $SrCl_2$).* ***Conclusion:*** *There was a significantly higher new bone formation ratio of mix 2 (25%Sr-75%HA) coated Cp-Ti implants than mix 1 (75% Sr-25% HA) coated Cp-Ti implant at 2 weeks healing period, also there was an increase in new bone formation ratio with time for both coated materials ($SrCl_2$) implants.*

Keywords: Strontium, Histomorphometric, Titanium, Bone

INTRODUCTION

One of the most important factors in the biological process of osseointegration is the surface of a synthetic implanted material since the fate of the implant is determined by biological mechanisms at the bone implant interface. The implant surface plays a role in process of osseointegration such as the biochemistry of bone formation, bone resorption, the regulatory mechanisms of osteogenesis and cellular response. Several studies focus on this to enable more rapid osseointegration of implants to reduce or even eliminate the period of bone healing for orthopedic and dental implant [1].

The implant topographical characteristics of the bone-implant interface is one of various lines of research to accelerate osseointegration process [2-7], another line which determine osseointegration is characteristics of the material used to make implant [8-10].

Implant surface treatment can be divided into methods where materials are added to the surface (e.g., titanium plasma spray and hydroxyapatite coating) and those where part of the surface is removed (e.g., microparticle blasting and acid etching) [11]. Strontium (Sr) is a trace element found in calcareous rocks and oceanwater [12], and a natural component of food and beverages [13]. Because of its similarity chemically and physically to calcium (Ca), also bone tissue content about 98% of the total body Sr because Sr natural bone-seeking element. [14,15].

Moreover, in various experimental studies and clinical trials studies indicated stable strontium ions (Sr) dual effect to promote bone formation and reduce bone resorption [16-18].

Strontium ranelate has positive effects on bone formation by enhancing the replication of preosteoblastic cells which form osteoblastic cells that are responsible for bone synthesis as show *in vitro* studies [19-22]. Strontium ranelate facilitates osteogenesis and phenotypic markers expression of in cultured osteoprogenitor cells or osteoblasts [16,23-25]. The net effects of strontium ranelate (i.e., reduction of osteoclast activity and promotion of osteogenic differentiation) thus distinguish the drug from the bisphosphonates (which act predominantly to inhibit bone remodeling) and intermittent PTH therapy (which acts to stimulate bone remodeling) [26].

The purpose of this study was to make histological and histomorphometric analysis of coated implant with strontium chloride ($SrCl_2$) mixing with hydroxyapatite (HA) at different concentration, in rabbit tibia at 2 and 6 weeks' implantation time.

MATERIAL AND METHODS

Grade 2 commercially pure titanium (Rod-shaped, 6 mm diameter) (supplied by GRS Titanium Ing.1550 Spruse Street Wooster, Ohio 44691 USA) were machined using Lathe machine in to 48 screw shaped implants with diameter 3.0 mm and length 8 mm (smooth part is 3 mm and threaded part is 5 mm).

By using ultrasonic bath, all implants were washed in acetone, alcohol, and deionized water in an, and then dried at 45°C.

Three electrolytes were prepared by mixing Sr-HA in different concentration. First electrolytes prepared by adding 3:1 strontium chloride to hydroxyapatite (75% $SrCl_2$ with 25% HA) to a liter of ethanol with 3.6 g Polyvinyl butyral (PVB), and second electrolyte was prepared by adding 1:3 strontium chloride to hydroxyapatite (75% HA with 25% $SrCl_2$) to a liter of ethanol with 3.6 g Polyvinyl butyral (PVB), Third electrolyte was prepared by adding 1:1 strontium chloride to hydroxyapatite (50% $SrCl_2$ with 50% HA) to a liter of ethanol with 3.6 g Polyvinyl butyral (PVB).

Electrophoretic deposition (EPD) process was done by application of different durations and different voltages for coating 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.

The solution was failed to coated screws at different voltage and time, so excluded from studies. The assessment surface topography of coated layer by using optical microscope (Nikon Type 120, Japan optical microscope) with digital camera type DXM 1200 F, then micrographs were analyzed through Nikon ACT- version 2.62, 2000 software.

Three healthy adult New Zealand rabbits from each group were used for histological testing. Rabbits were euthanized with overdose of anesthesia medication, then bone sectioning was performed by disc cutter with low rotating speed and vigorous cooling, a bone-implant block cut about 5 mm away from the head of the implant were immediately stored in 10% freshly prepared formalin and left for overnight for fixation.

Then bone implant block was divided horizontally using a sharp scalpel into two parts with cross section. Total of 12 sections (4-5 μm of thickness) were made for each coating material in each period of time.

Photographs of each section were taken by light microscope with SAMSUNG, GT-N7100 camera at x4 power magnification. The area of new bone was marked and measured using Image J software (NIH Image, National Institutes of Health, Maryland, USA).

The new bone was determined according to criteria stated by Shapiro, as the area which look like coarse meshwork (trabeculae bone) of pink tissue surrounding patches of much lighter or unstained tissue or matrix, the area of new bone was marked [27] calculated using the following formula [28]:

$$NBFR = \frac{Area\ of\ newly\ formed\ bone}{total\ tissue\ area} \times 100$$

The number of osteoclasts and osteoblasts within the bone of both coating materials were counted at a magnification of 40x. Osteoblasts were identified based on their morphology as being cuboidal, with plump, large nuclei, and localized on the bone or osteoid surface. Osteoclasts were identified as large multi-nucleated cells stained with eosin, containing, and located immediately adjacent to the bone surface.

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 as statistically significant for p-values <0.05.

RESULTS

All rabbits showed normal movement after one week which indicates that the rabbits tolerated the implantation, there was no sign of gross infection, tissue reaction. The screws could not be moved by manual force.

The mean value of new bone formation 2 weeks post implantation for implant coated with mix 1 (75% Sr-25% HA) was 45.7 ± 3.8 and for mix 2 (25% Sr-75% HA) was 141.6 ± 11.8 and after 6 weeks of implantation 33.7 ± 2.8, 49.8 ± 4.1 for the mix 1 (75% Sr-25% HA) and mix 2 (25% Sr-75% HA) coating of commercially pure titanium implants respectively. The significant difference between mix 1and mix 2 at 2 weeks interval and non-significant after 6 weeks implantation (Tables 1-4).

Table 1 Descriptive statistics and 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 weeks intervals

Descriptive Statistics								t-test for equality of means	
Time	**Groups**	**N**	**Minimum**	**Maximum**	**Mean**	**SE**	**SD**	**t-test**	**Sig.(2-tailed)**
2 weeks	Mix 1	12	1.01	6.1	3.8121	0.50156	1.73745	3.296	0.007
	Mix 2	12	1.85	23.62	11.8078	2.2174	7.68161		
6 weeks	Mix 1	12	0.9	5.58	2.809	0.48817	1.69107	1.343	0.206
	Mix 2	12	1.11	10.78	4.1519	1.07741	3.73226		

Table 2 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		p-value
		t	df	
Mix 1	2 weeks x 6weeks	1.433	22	0.166
Mix2	2 weeks x 6weeks	3.105	22	0.005

Table 3 Descriptive information on osteoblast numbers in the Mix 1 (75% Sr-25% HA) and mix 2 (25% Sr-75% HA) coated implants at 2 and 6 week intervals

Time	Groups	N	Minimum	Maximum	Mean	SE	SD
2 weeks	Mix 1	20	4	12	7.012	0.489	2.189
	Mix 2	20	8	27	18.18	1.251	5.595
6 weeks	Mix 1	20	6	15	10.3	0.528	2.364
	Mix 2	20	7	17	11.71	0.565	2.527

Table 4 Descriptive information on osteoclast numbers in the. Mix 1 (75% Sr-25% HA) and mix 2 (25% Sr-75% HA) coated implants at 2 and 6 week intervals

Time	Groups	N	Minimum	Maximum	Sum	Mean	S E	S. D
2 weeks	Mix 1	4	1.5	3	8.5	2.125	0.31458	0.62915
	Mix 2	4	1	2.75	7.55	1.8875	0.39284	0.78568
6 weeks	Mix 1	4	0.3	1.2	3.1	0.775	0.18428	0.36856
	Mix 2	4	0.4	0.6	2.1	0.525	0.04787	0.09574

DISCUSSION

The local application of strontium might be alternative method to systemic application to enhance implant osseointegration. in this study, advantage of strontium for inhibition of bone resorption and promotion of new bone formation was gained. Furthermore, direct contact of SrHA coatings to the surrounding bone could have a more direct effect on implant osseointegration than systemic administration of strontium.

Histological feature of Ti implant coated SR-HA mixing in rabbit tibia bone after 2 and 6 weeks of implantation showed new bone formation along surface of implant with solitary island of bone in bone marrow under light microscopy.

The new bone formation might be due to effect of the coating material which is in contact with bone. The action of HA starts by release of calcium and phosphate ions which is considered responsible of bioactivity of HA, this dissolution of calcium and phosphate ions is followed by precipitation of biological cap layer then, organic compounds are incorporated into this newly formed layer and cell like osteoprogenitor, osteoblast and osteoclast colonize this layer, therefore bone formation begin with action of these cells [29].

The action of strontium chloride depends on release of Sr ions that enhance initial bone response Strontium ranelate activates osteoblast replication and exerts protective effects on osteoblast apoptosis via CaSR-dependent- and CaSR-independent mechanisms. *In vitro,* strontium has been shown to exert a positive effect on the replication of osteoprogenitor cells and preosteoblasts at the same time as it increases the syntheses of collagen and non-collagen proteins in cells from the cranial vault and in mature osteoblasts in rats. In addition, strontium ranelate stimulates the differentiation of osteoblastic precursors or mature osteoblasts capable of intervening in bone mineralization. Strontium ranelate has also been capable of increasing the expression of critical genes in osteoblastic differentiation, such as Runx 2 and BSP (bone sialoprotein) [30].

Strontium (Sr) activates osteoblastogenesis by promoting osteoblastic cell replication and differentiation and reducing apoptosis by mechanisms involving activation of CaSR results in enhanced extracellular signal regulated kinases (ERK1/2), also through activating protein kinase C (PKC), Protein kinase D (PKD), and P38 mitogen-activated protein kinases (P38 MAPK) pathways. So, strontium activation of the CaSR in bone cells engages multiple downstream signaling pathways that control cell proliferation, differentiation, and survival. The effect of strontium on osteoblast does not rely only on CaSR but present another receptor (orphan receptor GPRC6A) which is similar to CaSR can activates ERK1/2. Strontium can induce apoptosis of osteoclasts via the CaSR but in a different manner than that which calcium stimulates the CaSR This, in turn, induces apoptosis of mature osteoclasts, which serves as a key step in the regulation of overall osteoclast activity and thereby in the process of bone resorption [30].

Since the coating layer is a combination of SR and HA therefore the action of both are clearly seen by new bone formation ratio.

The result suggested significant increase in NBA for mix 2 than one after 2 weeks of implantation, as previous study showed the effect of HA is delay 4 to 6 weeks, the action in this time depend only on Sr effect which show increase in NBFA in mix 2 than mix 1 which was statistically significant. There were two explanations, the first might be due to the high concentration of Sr in mix 1 solution and because too high density of strontium this may led to the formation of thick layer of coating and then stripping off from implant screw surface and loosing much of strontium ions from implant surface compared to mix which form reasonable layer thickness.

Another explanation is that EPD depend on more ion moving between both cathode and anode by different in electropotensional also so more power need to more Sr ion than HA ions due to different in density and particle size between HA and Sr and different of concentration in both mix.

Also, after 2 weeks show increase in number of osteoblast and decrease number of osteoclast in mix 2 than mix 1 which due to dual effect of sr, which agree with Capuccini, et al. [31], which found that cultured 3%, 5% and 10% of SrHA mixture and HA alone, the result showed osteoclast number in all SrHA mixture sample was significantly less than HA alone and it decrease in number with increasing strontium percentage.

A result showed that osteoclast number in all SrHA samples was significantly less than on HA alone, and it decreased with increasing strontium percentage. Similar results of osteoclast number were observed which agree with result of this study show increase in number of osteoblast and decrease in number of osteoclast in mix 2 more than mix 1.

After 6 weeks there was non-significant difference between mix 1 and mix 2 which may be due to the mix contain strontium which has depend effect so with time and due to release from coating layer, so its effect also reduces, and action more depend on effect HA this time.

Both Sr and HA coatings showed increased ratio of bone formed by time HA as this process is normal physiological process, Robert, et al. 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”.

CONCLUSION

There was significantly higher new bone formation ratio of mix 2 (25% Sr-75% HA) coated Cp-Ti implants than mix 1 (75% Sr-25% HA) coated Cp-Ti implant at 2 weeks healing period, also there was increased new bone formation ratio with time for both coating materials ($SrCl_2$) implants.

DECLARATIONS

Conflict of Interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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