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Implantation and *In Vivo* study of the biogenic hydroxyapatite- coated Ti/Al alloy in dogs

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Abstract

Aim: The main goal of the study is the evaluation of the toxicity effect of the implantation of Ti/Al alloy coated with HA on the wound healing as well as the biological effects and safety evaluation in dogs.

Methods: fifteen cylindrical, one-stage implants of Ti/Al alloy substrate with a 32.49 µm thick plasma-sprayed HA coating were performed and implanted in fifteen mongrel male dogs. The dogs were randomly divided into three groups, the control group, which left without implantation, the other two groups are group with implanted Ti/Al alloy rods coated with HA implanted for 2 weeks and group with implanted Ti/Al alloy rods coated with HA implanted for 4 weeks. The wound healing, biological activity, inflammatory effect, and toxicity effect for the dogs having Ti/Al alloy coated with HA implants were evaluated

Results: The in vivo study results signalized new skin layers formation and complete healing of the skin in all implanted samples after one month of implantation in dog's skins (epidermal layer). The operation results were confirmed by histological analyses and blood analyses, which obviously indicate that there are no side effects or toxicity even after one month of implantations.

Keywords: Ti/Al alloy coated with HA, In vivo study, Histological analysis, Blood analysis

Introduction

Titanium and its alloy are characterized by their good mechanical properties as well as their adequate biocompatibility, which candidates them for using for bone and dental substitution [1]. To improve the alloys biocompatibility, ceramic biomaterials in particular hydroxyapatite (HA) were applied as coating materials for the surface of the alloys. HA coating enhanced the capability of the bone-bonding between the bones and the implant [2,3]. The most abundant inorganic constituent in human bones and teeth is HA [Ca_{10} $(PO_4)_6(OH)_2$] [4]. Since a long time, HA was applied as a coat for monitoring and enhancing the osseointegration among the human bones and the implants [5,6].

Metals are coated with HA via numerous coating techniques like sputtering, suspension plasma spray, micro-plasma spray, sol-gel pulsed-laser, electro-deposition, hydrothermal electrochemical method, etc [7-10]. Amongst these coating methods, the plasma-sprayed technique is chosen to be the most

utilized deposition technique of HA coating on metals due to its speedy and cheap [8, 11, 12].

The steps for the healing of the wound after the implantation of Ti/Al alloy in the living body is divided to 1) hemostasis, which considered the first step in the wound healing where the blood plasma and cells are found on all sides of the implant. 2) formalization of a matrix of fibrin enriched with platelets, white cells and erythrocytes. 3) After two weeks it is the time for macrophages to form from blood monocytes near to the implant exterior [13]. The tissue restoration improvement has been noticed via the boost in the extracellular matrix (ECM) synthesis, cells discrimination and reproduction. It mostly occurs after 3-4 days of implantation as soon as the tissue starts to endearment and vascularize templet is composed [14]. After 2 weeks pluripotent mesenchymal stem cells differentiate and finally mineralized [15].

The biomaterial implants possess great efficiency in bone repairing. While the surface of the biologically inert materials is treated by the living body as unwanted, it is not the case with the biomaterials since they are speedily merged with the bones by the direct contact repair. The direct contact permits the formation of a chemical bond with the contacting bone, which in turn organizes a lamellar bone immediately in the site. Then and before the new bone is formed the lamellar bone is abstracted by osteoclasts [16].

The present study aims at the estimation of the effect of Ti/Al alloy coated with HA implantation on the wound healing, biological activity, inflammatory effect, and toxicity effect for the dogs having Ti/Al alloy coated with HA implants.

Materials and Methodology

a. Material

Fifteen cylindrical, one-stage implants of Ti/Al alloy substrate with a 32.49 µm thick plasma-sprayed HA coating were performed. The total length of each implant was 13.00 mm and the diameter was 4.00 mm.

b. Methods

The present study was approved by and in accordance with the rules of the ethical committee at National research Centre, and the Animal Use and Care ethical Committee at Faculty of Veterinary Medicine, Cairo University, Egypt.

Fifteen mongrel male dogs, aged 1-3 years and weighted 15-25 kg were included in this study. Induction of severe acute pancreatitis was carried out in all dogs. Then the dogs were randomly divided into 3 groups (5 dogs each) according to the treatment. The control group (group I) was left without implantation (with wounded skin only). While (group II) is the group of the implanted Ti/Al alloy rods coated with HA implanted for 2 weeks. Implanted Ti/Al alloy rods coated with HA implanted for 4 weeks was assigned as (group III). The statistical analysis was used SPSS program (independent (t) test) for comparing between the implanted Ti/Al alloy rods coated with HA group and the control group. The results are the mean values of 5 dogs ± Stander divination (SD).

General Anesthesia

All dogs were premedicated with subcutaneous injection of atropine sulphate 0.05 mg kg^{-1} body weight (Atropine Sulphate: ADWIA Co., Cairo, Egypt) and intramuscular Xylazine HCl 1.1 mg kg^{-1} body weight (Xylaject: ADWIA Co., Cairo, Egypt). The anesthesia was induced by intravenous Ketamine HCL 5 mg kg^{-1} body weight (Keiran: EIMC pharmaceuticals Co., Cairo, Egypt). Maintenance of anesthesia during the operation was done with 25 mg kg^{-1} incremental doses of 2.5% solution of Thiopental sodium given intravenously (Thiopental sodium: EIPICO, Cairo, Egypt).

Creation of Skin Wounds and Ti/Al alloy coated HA implantation

The skin wounds in dogs were carried out, and stitched according to the steps described by Abu-Seida [17]. Cefotaxime sodium (10mg/ kg) and diclofenac sodium (1.1mg/ kg) were offered once a day for 5 days post-surgery for pain and infection control. These wounds were divided randomly into three equal groups (5 each) including group I (control group), group II (Ti/Al alloy coated HA group implanted for 2 weeks) and group III (Ti/Al alloy coated HA group implanted for 4 weeks). The wounds were cleaned and dressed twice a day until wound healing. Measurement of the wound area (cm^2) was monitored

planimetrically at 1, 3, 7, two weeks and one month after injury (DAI) according to the method described by Oryan et al [18].

Biochemical studies for the Evaluation the Safety of Implanted Materials.

The dogs were followed up by biochemical tests including; Liver Function Tests, Kidney Function Test, Measurement of Tumor Markers, Inflammatory Effect of the Implant, Measurement of Free Radical Biomarker, the study of the toxicity effect of the implant on RBCs, WBCs, and Hemoglobin levels on normal dogs, also after 1 day, 3 days, 7 days, 2 weeks and one-month post-surgery.

Histological examinations

The samples needed for the histological examination were collected and prepared according to the steps described by Banchroft et al [19].

Results and Discussion

Biological studies for safety evaluation of implanted materials

1. Liver function tests

a. Determination of serum alanine aminotransferase (ALT, u/l) activities.

Table (1) represents alanine aminotransferase (ALT) activities in serum of dogs with Ti alloy implant together with those of the control group. the statistical analysis was carried out to evaluate the difference between ALT activities in serum of implanted groups and the control group. Up to 2 weeks, the statistical analysis indicated a decrease of ALT activity compared to that of the control group, but statistically, such decrease was not significant ($p > 0.05$). After 4 weeks of the operation ALT activities in serum of implanted groups was slightly increased in comparison to its level in the control group but the increase was still insignificant and its value was ($p = 0.63$). This decrease is because of the metabolic changes and the decomposition of the implanted material; the normal ranges of ALT activity for dogs is (5-107 u/ l) [20].

Table 1. ALT of the implanted dogs in comparison with control group

Time, days	Control group	Ti/Al alloy coated HA	Sig. value
0	52.00 ± 0.019	46.00 ± 2.54	0.36
1	59.00 ± 0.20	53.00 ± 2.98	0.44
3	55.90 ± 0.02	46.80 ± 3.29	0.10
7	49.60 ± 0.40	48.00 ± 0.32	0.32
14	52.00 ± 0.32	40.40 ± 0.03	0.08
28	44.90 ± 1.16	45.80 ± 0.80	0.63

b. Measurements of serum Aspartate Aminotransferase (AST, u/l) Activities

Up to 7 days the AST activity of the group II and III dogs were less than those of the control group, Table 2. After 2 weeks up to four weeks, the AST activity was slightly increased in comparison to the control group. Statistical analysis results showed that the difference between aspartate aminotransferase (AST) activities in serum of group II dogs compared to that of control group was not significant $p \geq 0.05$ ($p = 0.49$). Normal range of AST activity for dogs is [5-55 u/l] [20].

Table 2. AST of the implanted dogs in comparison with control group

Time, days	Control group	Ti/Al alloy coated HA	Sig. value
0	24.19 ± 0.16	24.75 ± 11.39	0.17
1	30.90 ± 0.20	28.66 ± 13.99	0.20
3	30.00 ± 0.40	25.60 ± 400	0.21
7	29.02 ± 0.02	24.20 ± 0.19	0.07
14	21.50 ± 0.41	23.50 ± 0.44	0.08
28	20.18 ± 0.199	21.56 ± 0.47	0.49

c. Measurements of serum alkaline phosphatase (ALP, u/l) activities:

Table 3. shows alkaline phosphatase (ALP) activity in serum of dogs implanted with Ti-alloy rods coated with HA as compared with the control group. It is clear from the table that ALP activity was lower in the serum of grafted animals. However, statistical analysis showed that the difference between (ALP) activity in serum of grafted animals and that of the control group was not significant $p \geq 0.05$ and the difference value was $p = 0.06$. Normal range of ALP for dog's is [20-200 u/l] [21].

Table 3. ALP of the implanted dogs in comparison with control group

Time, days	Control group	Ti/Al alloy coated HA Group	Sig. value
0	29.29 ± 0.21	27.80 ± 7.49	0.43
1	31.99 ± 0.02	29.86 ± 7.77	0.11
3	42.00 ± 0.02	35.30 ± 0.153	0.07
7	45.02 ± 0.002	34.60 ± 0.40	0.09
14	50.14 ± 0.24	45.30 ± 0.09	0.05
28	31.54 ± 0.23	26.92 ± 2.31	0.06

Liver function tests showed that the grafted animals had normal liver function as compared to the control group. This could indicate that the degradation products of the Ti-alloy coated with HA did not cause liver disorder.

2- Kidney function Tests

a. Determination of serum creatinine activities

Table 4. shows the serum creatinine levels of animals implanted with Ti/Al alloy coated with HA in comparison to the control group. The statistical analysis indicated that there is difference between the control groups and the implanted dogs but this difference is not significant (p = 0.68). Normal range of dogs' creatinine is [0.5-1.6 mg/dl] [20].

Table 4. Serum creatinine, mg/dl of the implanted dogs in comparison with control group

Time, days	Control group	Ti/Al alloy coated HA	Sig. value
0	1.10 ± 0.002	0.87 ± 0.72	0.50
1	0.89 ± 0.002	0.95 ± 0.06	0.10
3	1.20 ± 0.004	0.99 ± 0.11	0.08
7	0.90 ± 0.001	0.60 ± 0.003	0.14
14	1.11 ± 0.006	0.80 ± 0.06	0.15
28	0.91 ± 0.005	0.80 ± 0.08	0.68

b. Measurements of serum urea level:

Table 5. shows the serum urea level of the animals implanted with Ti/Al alloy coated with HA in comparison to the control group. The results showed that in general the serum urea level was decreased for the implanted dogs compared to the control group. The statistical analysis indicated that there is difference between the control groups and the implanted dogs but this difference is not significant (p = 0.84). Normal range of urea level for dogs is [8.7- 30.5 mg/dl] [20].

The results indicated that the implanted dogs have normal kidney function as the control group. This proves that the degradation products of Ti/Al alloy coated HA did not cause any kidney failure

Table 5. Serum urea level (mg/dl) of the implanted dogs in comparison with control group

Time, days	Control group	Ti/Al alloy coated with HA	Sig. value
0	24.99 ± 0.02	26.60 ± 2.20	0.30
1	25.00 ± 0.20	27.00 ± 2.40	0.45
3	36.60 ± 0.04	27.60 ± 3.76	0. 17
7	27.90 ± 1.20	28.00 ± .06	0.07
14	29.80 ± 1.06	29.00 ± 1.12	0.18
28	30.60 ± 0.40	30.00 ± 1.91	0.84

Measurement of tumor markers

Measurement of α-L-Fructosidase (AFU) activity in serum

Table 6. shows that the α-L-Fructosidase (AFU) activity in the serum of dogs implanted with Ti/Al alloy coated with HA was lower on comparing with that of the control group. Statistical analysis showed that the grafted materials have no carcinogenic effect over the implanted dogs as compared with the control group. It has been recorded that the difference between AFU activity in the serum of the implanted dogs and that of the control group was not statistically significant $p \geq 0.05$ (P=0.17).

Measurement of Arginase activity in serum

Table 7. shows the arginase activity in serum of the dogs implanted with Ti/Al alloy coated with HA as compared with the control group. It was noticed that after one day the arginase activity in serum of the grafted animals was increased on comparison to the control group. From the third day up to 4 weeks, the arginase level for the implanted dogs started to lower than the control group. However, statistical analysis indicates that the difference between arginase activity in serum of the implanted dogs and the control group was not statistically significant $p \geq 0.05$ (0.77).

Table 6. alpha-L-fructosidase (u/l) of the implanted dogs in comparison with control group

Time, days	Control group	Ti/Al alloy coated with HA Group	Sig. value
0	0.85 ± 0.002	0.800 ± 0.52	0.48
1	1.40 ± 0.002	1.30 ± 0.23	0.08
3	1.51 ± 0.006	0.88 ± 0.17	0.07
7	0.79 ± 0.004	0.50 ± 0.005	0.92
14	0.86 ± 0.02	0.79 ± 0.06	0.30
28	0.81 ± 0.009	0.68 ± 0.09	0.17

Table 7. Arginase level (u/l) of the implanted dogs in comparison with control group

Time, days	Control group	Ti/Al alloy coated HA groups	Sig. value
0	5.40 ± 0.002	5.50 ± 0.04	0.42
1	2.90 ± 0.002	4.10 ± 0.09	0. 39
3	10.08 ± 0.04	8.24 ± 0.08	0.07
7	3.24 ± 0.40	3.27 ± 0.40	0.08
14	6.92 ± 0.57	4.63 ± 0.08	0.40
28	8.28 ± 0.18	8.25 ± 0.17	0.77

The reduced Glutathione (GSH) measurement

Table 8. shows the reduced Glutathione (GSH) level in the dog's serum. The table indicates a slight decrease in the implanted dogs' GSH level in the serum is very slight. During the remaining period of the experiment (up to 4 weeks), the decrease in the GSH level was noticeable. The results are statistically insignificant $p \geq 0.05$ (0.30).

Table 8. The tumor markers (GSH) level of the implanted dogs in comparison with control group.

Time, days	Control group	Ti/Al alloy coated HA group	Sig. value
0	14.89 ± 0.002	15.40 ± 0.79	0.37
1	18.69 ± 0.008	18.22 ± 1.40	0.08
3	13.96 ± 0.02	13.30 ± 0.06	0.055
7	19.00 ± 0.008	15.59 ± 0.38	0.08
14	17.88 ± 0.02	16.70 ± 0.06	0.09
28	19.24 ± 0.40	14.26 ± 1.20	0.30

The results of the tumor markers cleared that the implanted materials have no carcinogenic effect.

Measurement of free radical biomarker

a. Super-oxide dismutase (SOD**)**

The SOD level in the serum of the dogs, who have a rod of Ti/Al alloy coated HA implanted in their bones is higher than that for the control group, Table 9. However, the statistical analysis indicates that the difference between SOD activity in the serum of implanted dogs and that of the control group is not statistically significant $p \geq 0.05$ (P=0.39). The noticed increase in the implanted dog's serum may be due to the metabolic changes and the self-defenses against the foreign bodies.

Table 9. The super-oxide dismutase (SOD) of the implanted dogs in comparison with control group, (U/mL)

Time, days	Control group	Group of Ti/Al alloy coated with HA	Sig. value
0	5.10 ± 0.002	4.78 ± 0.22	0.34
1	3.80 ± 0.002	4.15 ± 0.02	0.07
3	2.91 ± 0.004	4.42 ± 1.15	0.08
7	3.90 ± 0.02	4.36 ± 0.16	0.06
14	4.49 ± 0.23	4.51 ± 0.009	0.59
28	5.18 ± 0.037	6.12 ± 0.94	0.39

b. lipid peroxides (LIP) (Malondialdehyde)

The results of lipid peroxides (LIP) in the serum of the dogs are shown in Table 10. It was noticed that the results for the LIP content for the serum of the implanted dogs are lower in comparison to the control group. But there is no significant difference (p= 0.18).

Measurement of nitric oxide and cortisol production in serum as an indicator of the inflammatory effect of implanted materials.

The results indicated that the level of nitric oxide (NO) of the implanted animals displayed a high nitric oxide activity until 2 weeks then decrease than control group. But this inflammation is normally and not significant (p=0.88), Table 11. On the other hand, the level of cortisol showed some inflammation during the first 2 weeks, but after 4 weeks the implanted dogs showed a cortisol level less than the control group, Table 12. The results are statistically insignificant (p=0.88 and 0.08 respectively).

Table 10. The lipid peroxides (LIP) of the implanted dogs in comparison with control group, (nmol/ ml).

Time, days	Control group	Group of Ti/Al alloy coated with HA	Sig. value
0	4.90 ± 0.002	5.91 ± 0.60	0.10
1	6.18 ± 0.02	6.05 ± 0.37	0.08
3	1.51 ± 0.005	0.88 ± 0.17	0.07
7	0.79 ± 0.004	0.50 ± 0.005	0.92
14	0.86 ± 0.016	0.79 ± 0.06	0.31
28	0.81± 0.009	0.68 ± 0.09	0.18

Table 11. The nitric oxide level of the implanted dogs in comparison with control group, (nmol/ l).

Time, days	Control group	Ti/Al alloy coated HA groups	Sig. value
0	16.70 ± 0.01	17.44 ± 0.71	0.12
1	18.90 ± 0.20	18.94 ± 0.87	0.05
3	15.10 ± 0.006	18.82 ± 0.17	0.07
7	16.00 ± 0.007	18.80 ± 0.49	0.09
14	14.60 ± 0.75	20.40 ± 0.40	0.19
28	12.00 ± 0.55	17.20 ± 0.49	0.88

The study of the toxicity effect of the implant on RBCs, WBCs, Hemoglobin levels and PLT counts after 1 day, 3 days, 7 days, 14 days and one-month post-surgery.

Table (13) shows the effect of Ti/Al alloy coated with HA on the RBCs, WBCs, and Hemoglobin levels, and the difference between control dogs and normal dogs implanted with Ti/Al alloy coated with HA, 1-day post-surgery. The table cleared that there is an increase in the RBCs, WBCs and Hb levels. On the other hand, PLT counts showed a decrease in its level. The abovementioned increase and decrease are not noticeable and the implanted doges showed normal levels, which indicate that the implantation has no effect on the measured parameters and the complete blood count (CBC) is normal [20-22].

Table 12. The cortisol level of the implanted dogs in comparison with the control group, (nmol/ l).

Time, days	Control group	Ti/Al alloy coated HA groups	Sig. value
0	3.61 ± 0.002	3.06 ± 0.21	0.15
1	2.40 ± 0.002	2.56 ± 0.06	0.05
3	2.18 ± 0.004	2.46 ± 0.03	0.069
7	2.10 ± 0.003	2.92 ± 0.01	0.08
14	3.63 ± 0.05	3.73 ± 0.07	0.12
28	3.56 ± 0.025	2.02 ± 0.29	0.08

Table 13: The toxicity effect of used implant on RBCs, WBCs, Hemoglobin and platelet counts, 1-day post-surgery.

Samples symbol	RBCs ($nx10^6$)	WBCs($nx 10^3$)	HB, g/dl	PLT ($nx10^5$)
Control group	7.24 ± 0.052	10.14 ± 0.215	15.66± 0.040	39±0.948
Ti/Al alloy coated with HA group	7.45 ± 0.46	15.70 ± 1.59	16.48± 0.962	36±0.684

Table 14. Indicates an increase in both the RBCs and HB levels, while the WBCs level and the PLT count was decreased for the implanted dogs after 3-days of the surgery. There is no significant effect on the measured parameters and the complete blood count (CBC) is normal.

Table 14: The toxicity effect of used implant on RBCs, WBCs, Hemoglobin and platelet counts, 3-days post-surgery.

Samples symbol	RBCs ($nx10^6$)	WBCs ($nx10^3$)	HB, g/dl	PLT ($nx10^5$)
Control group	7.75 ± 0.09	17.14 ± 0.46	17.50 ± 0.14	150 ±0.45
Ti-alloy coated with HA group	7.81 ± 0.19	12.60 ± 0.25	17.60 ± 0.36	140±0.39

Table 15 clearly that after 7 days the implanted dogs' blood behaves similar to those of 3-days. Both the RBCs and Hb level of the implanted dogs are higher than those for the control group, while the WBCs level and the PTL count are less in comparison to the control group. In general, the four measured parameters normally behaved and there is no significant effect on four parameters measured.

Table 15: The toxicity effect of used implant on RBCs, WBCs, Hemoglobin and platelet counts, 7-days post-surgery.

Samples symbol	RBCs ($nx10^6$)	WBCs ($nx10^3$)	HB, g/dl	PLT ($nx10^5$)
Control group	7.39 ± 0.040	16.2 ± 0.200	16.6 ± 0.09	160 ± 0.008
Ti/Al alloy coated with HA group	8.20 ± 0.74	8.4 ± 0.43	18.4 ± 0.04	150 ± 0.004

The results shown in table (16) pointed out to an approximately no change in the RBCs level after 7-days of implantation. On the other hand, a noticeable decrease in WBCs was observed. Such behavior is due to the defense mechanism of the animal's body against the implanted materials as a primary stage of normal healing. The observed increase of Hb level and decrease of PLT counts is due to the need of the animal body to more time for complete healing of the open area of the wound [23].

Table 16: The toxicity effect of used implant on RBCs, WBCs, Hemoglobin and platelet counts, 14-days post-surgery.

Samples symbol	RBCs ($nx10^6$)	WBCs ($nx10^3$)	HB, g/dl	PLT ($nx10^5$)
Control group	7.94 ± 0.06	16.20 ± 0.003	17.60 ± 0.05	34.00 ± 0.00
Ti/Al alloy coated with HA group	7.44 ± 0.54	7.50 ± 0.93	16.50 ± 0.06	70.00 ± 0.008

The results displayed in Table (17) showed a slight change in the RBCs, WBCs and HB levels for the implanted dogs, while the PTL count is the only parameter that increased. The increase in the PTL counts is within the normal range for the dogs as the normal ranges for dogs blood analysis is Hb 12-20.3 g/dl [20], RBCs 4.8-9.3 $x10^6$ [22], WBCs 6-17.50 $x10^5$ ul [22] and PLT 170-400 [21]. The abovementioned results proved that the implantation of the Ti/Al alloy coated with HA has no toxicity effect on the doges and that the healing of the implanted dogs was faster in comparison to the control group. In addition, there were no side effects or toxicity were reported post-surgery. Accordingly, the implanted material may be suggested for the healing of different types of animals' wounds.

Table 17: The toxicity effect of used implant on RBCs, WBCs, Hemoglobin and platelet counts, 28-days post-surgery.

Samples Symbol	RBCs ($nx10^6$)	WBCs ($nx10^3$)	HB, g/dl	PLT ($nx10^5$)
Control group	8.09 ± 0.082	15.70 ± 0.001	14.70 ± 0.05	170.00 ± 0.01
Ti/Al alloy coated with HA group	8.47 ± 0.03	14.00 ± 0.005	15.50 ± 0.007	200.00 ± 0.05

1- Histopathological Findings

1- Control group

a- After two weeks:

The epidermal layer showed focal acanthosis in the prickle cell layer (Fig1) associated with granulation tissue formation in the underlying dermis (Fig.2). Focal inflammatory cells infiltration was detected in the dermis and subcutaneous tissue (Fig.3). It is well known that inflammation happens as part of the recovery process. Inflammatory cells, like neutrophils and monocytes, enlist within a short time (ranging from minutes to hours) into the implant position. severe inflammation persists then 2-3 days when monocytes bit by bit distinguished to macrophages that reside in the healing site for up to a couple of weeks. Tissue healing is monitored by the increase in cell distinguishing and proliferation and extracellular matrix (ECM) synthesis [14].

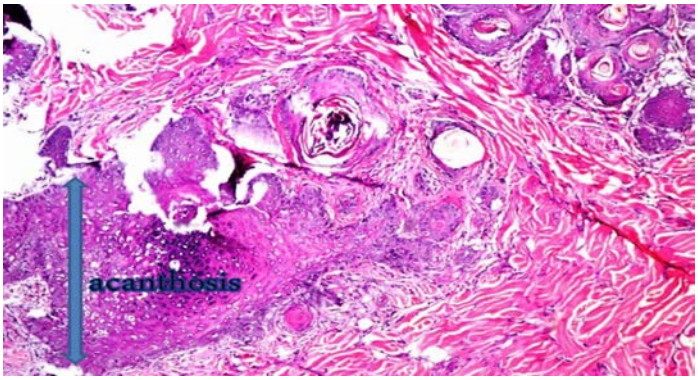

Fig. 1. The skin of a control group dog showing focal acanthosis after two weeks of pre-healing in the prickle cell layer of the epidermis (H&E 16).

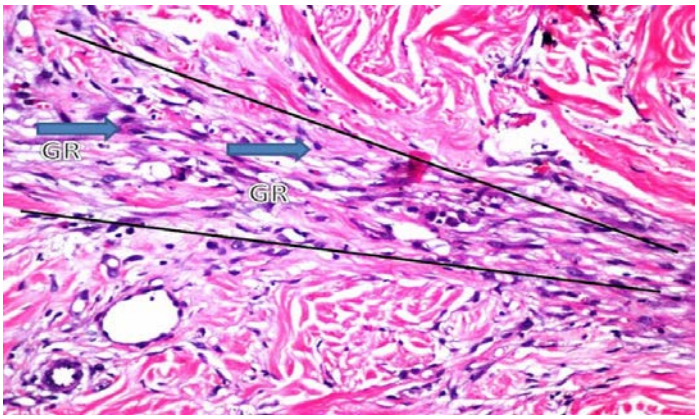

Fig. 2. The skin of the dog of the control group, showing granulation tissue (G) formation in the dermis underneath the acanthosis (H&E 40).

a- After four weeks

Focal area of granulation tissue was detected in the dermis (Fig.4), associated with focal inflammatory cells aggregation in the dermis as well as in the subcutaneous tissue. A focal haemorrhage in the dermis was observed (Fig.5).

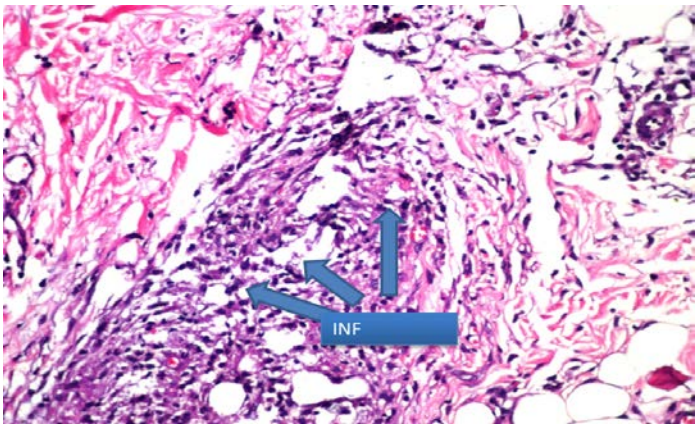

Fig. 3. The skin of the dog of the control group showing focal inflammatory cells (INF) infiltration in the dermis and subcutaneous tissue (H&E 40).

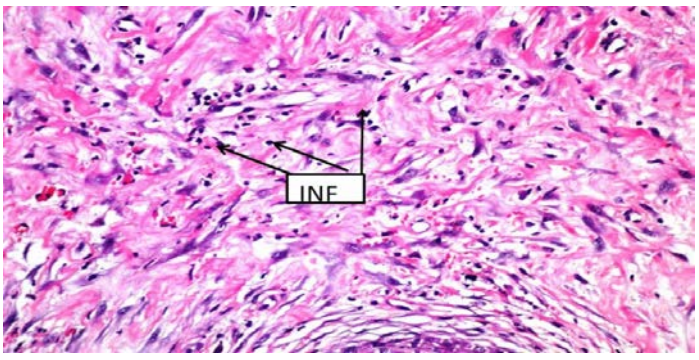

Fig. 4. The skin of a dog of the control group after four weeks, showing a focal area of granulation tissue formation associated with focal inflammatory cells in the dermis and subcutaneous tissue (H&E 40).

2- Group II (Ti/Al alloy coated HA GROUP):

a- After two weeks

The dermal layer showed a focal area of necrosis with extravasated red blood cells and inflammatory cells infiltration (Fig. 6, 7).

b- After four weeks

Figure (8) shows the skin of a dog of group II after 4 weeks showing the complete healing of the skin (identify granulation tissue formation of a fibroblasts and angioblasts) (H&E 40).

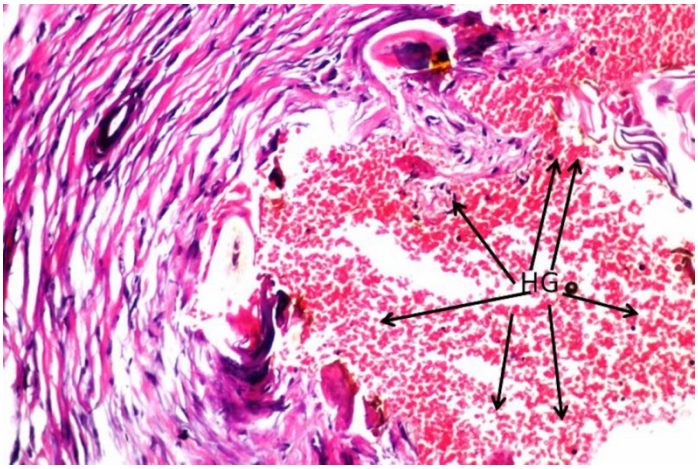

Fig. 5. Skin of a dog of the control group after four weeks, showing the focal area of inflammatory cells in aggregates in the dermis and subcutaneous tissue. The figure identified the focal haemorrhages (H) in the dermis (H&E 40).

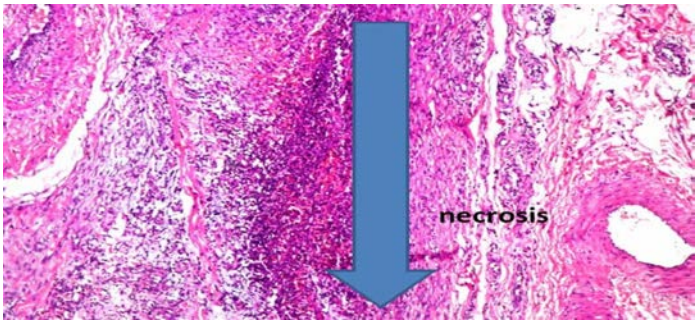

Fig. 6. Skin of a dog of Ti-alloy coated HA group II after 2 weeks showing focal necrosis with extravasated red blood cells and inflammatory cells infiltration area of inflammatory cells in the dermis (H&E 16).

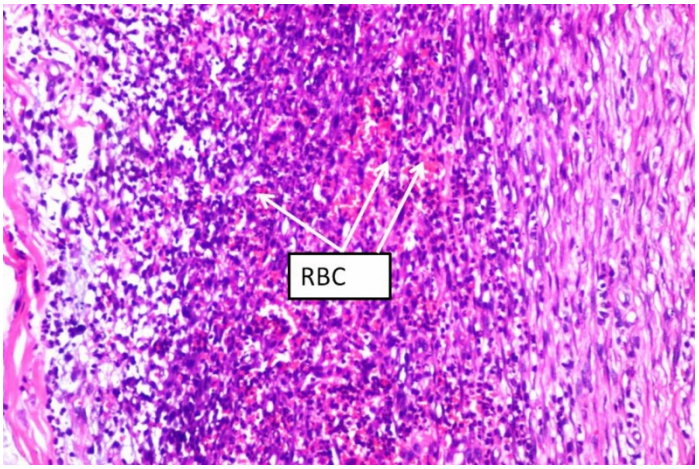

Fig 7. Skin of a dog of group II after 2 weeks showing the magnification of (Fig.6) to identify the necrosis and massive inflammatory cause infiltration in the dermis (H&E 40).

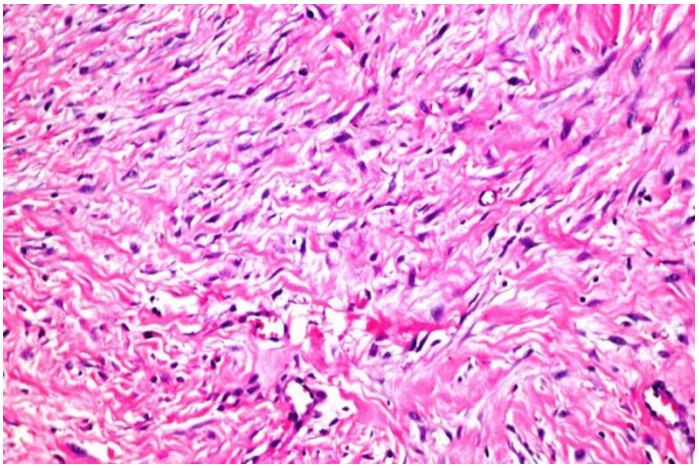

Fig. 8. Skin of a dog of group II after 4 weeks showing the complete healing of the dog' skin

Conclusion

1. As a conclusion, it was found that Ti/Al alloy coated with HA helping in the process of healing in the dog's skin. The healing was achieved within one month.
2. The degradation products of Ti/Al alloy coated with HA implanted in dogs did not cause any liver disorder or kidney failure. Both the liver and kidney functions of the dogs were normal.
3. The results of the tumor markers cleared that the implanted materials have no carcinogenic effect. It also showed that the implantation has no effect on the measured parameters and the complete blood count (CBC) is normal.
4. Additionally, the results indicated that there were no side effects or toxicity were reported post-surgery.

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