

After surgery the health of rabbits beside the site of operation checked daily for any infections and complication. The animals were under superintendence for 2 weeks and 6 weeks.

Biomechanical (Push out bond strength Test): according to the design of study the Rabbits were sacrificed after 2 and 6 weeks' time intervals. Right and left femurs with implant specimens were dissected and all flesh was removed. On the same day as euthanasia, push out bond failure was done by using an Instron universal testing machine [18].

The clearance hole was made below the implant specimen at least 3.5 mm in diameter to record the pure force required for the implant/bone bond breakage, the femur was fixed in a clamp for support the bone, and the clamp was fixed in the universal testing machine as shown in (Figure 1). The specimen was loaded at a rate of 1 mm/min, load was applied to the implant specimen through a specially designed plunger, with cylindrical working head 3 mm in diameter, connected to the crosshead of the universal testing machine, The maximum load of failure was recorded in Newton (N), the apparent shear stress was obtained from dividing the maximum load on the contact area which was the periphery of cylindrical implant specimen [19].



Figure 1: Instron universal testing Machine with bone/implant block.

Histomorphometric analysis: It includes calculating the areas of the thickness of the bone trabeculae tissue around the implant bar and bone implant contact BIC for all the groups. Rabbits were scarified for each healing period 2 and 6 weeks for histomorphometric examination by overdose aesthetic solution according to [16].

Bone implant block was prepared by cutting the femur bone about 5 mm away from the implant and then they stored immediately in 10% newly freshly prepared buffered formalin for fixation [15].

Preparation of the specimens: This step include the following

- **Fixation:** The specimens were immediately fixed in 10% freshly prepared neutral buffered formalin for 3 days.

- **Decalcification:** The specimens were left in formic acid-sodium citrate solution which was prepared freshly from 2 solutions:
- Solution A: 125 cc formic acid 90%. 125 cc distilled water.
- Solution B: 50 mg sodium citrate. 250 cc distilled water.

After that the two solutions were mixed and the specimens put in it, to have decalcification of the bone the solutions were changed every 3 days.

Decalcification of the bone was checked using a narrow needle. The bone was considered to be decalcified when the needle could penetrate to the deepest part of the bone in the sockets blocks.

Washing the specimens with tap water

Dehydration: The specimens were dehydrated by passing them through a series of increasing of alcohol concentration (40%, 60%, 80%, 95%, and absolute alcohol). Then the specimens were passed through two jars of xylene, each jar for half an hour.

Embedding: The specimens were placed in a dish of melted embedding paraffin and the dish was put into a constant temperature oven regulated about 53-60°C.

During the course of several hours, the specimen was changed to two successive dishes of paraffin so that all of the xylene in the tissue was replaced by paraffin (each dish for one hour). The specimen was placed in the centre of block paraffin.

Sectioning: Five μm -thick semi serial cross sections of the implant site were mounted on clean glass slides for routine haematoxylin and eosin staining (H and E).

Haematoxylin and eosin stain

The obtained sections were dewaxed with xylene and dehydrate in descending alcohol concentration.

- Stained with Mayer's haematoxylin stain for 7-10 min.
- Washed in tap water 1-5 min.to remove the excess stain
- Stained with eosin for 1-2 min.
- Dehydrated in absolute alcohol for 2-3 min. and clear with xylene.
- Cover slips were fixed on stained tissues using D.P.X.

Examined under the light microscope for histomorphometric. Histomorphometric findings were estimated by means of three characteristics in six quadrangular sections measuring 200 × 200 micro meters around the implant site.

BTT: the thickness of the bone trabeculae tissue around the implant bar; BTT was measured by means of linear measurements perpendicular to bone determined on every aspect of the implant bed sites [20].

BIC: Bone-to-implant contact (%) was assessed by manually measuring the relative length of bone tissue in direct contact with the implant. The measurements from

both sides of the implant in three different sections were averaged and used for statistical analysis [21].

values at both 2 weeks and 6 weeks than both Ti₆Al₇Nb and CPTi.

Statistical analysis

The appropriate statistical method was followed to analyse the results by using Statistical Package for Social Sciences (SPSS) version 24.

The difference was statistically significant with p-value <0.001 for both weeks.

RESULTS

Biomechanical push out bond strength test

The results of pushout bond strength test in Table 1 recorded that max phase alloy had the highest mean

Table 1: Push out test for CPTi, Ti6Al7Nb and Ti2AlC at both 2 weeks and 6 weeks.

Time	Material	Mean	ANOVA test	
			F	Sig.
Two Weeks	Max	10.425	157.612	0
	TiAl ₇ Nb	7.763		
	CPTi	3.144		
Six Weeks	Max	18.172	127.641	0
	TiAl ₇ Nb	9.42		
	CPTi	11.784		

In Table 2 pairwise analysis using Bonferroni showed that there was a high statistically significant difference for max phase alloy with Ti₆Al₇Nb and CPTi, respectively also for both weeks.

Likewise, Ti6Al7Nb had higher mean value than CPTi with P-value of 0.001 at both 2 weeks and 6 weeks.

Table 2: Bonferroni pairwise analysis between each two material at 2 and 6 weeks' time interval.

Time	(I) Material	(J) Material	Mean Difference (I-J)	Sig.
Two Weeks	Max	TiAl ₇ Nb	2.6620*	2.146 E-6
		CPTi	7.2810*	8.221 E-16
	TiAl ₇ Nb	CPTi	4.6190*	4.082 E-11
Six Weeks	Max	TiAl ₇ Nb	8.7520*	1.906 E-14
		CPTi	6.3880*	3.077 E-11
	TiAl ₇ Nb	CPTi	-2.3640*	0.001

Similarly, there was a higher mean values for the pushout test at 6 weeks than 2 weeks for CPTi, Ti6Al7Nb and Ti2AlC as shown in Table 3.

Also for the differences between 2 and 6 weeks. There was a high statistically significant difference for all of them with p-value<0.001.

Table 3: Push out bond strength test at both 2 weeks vs 6 weeks for CPTi, Ti6Al7Nb and Ti2AlC.

Material	Time	Mean	Std.	F	Sig.
Max	2 weeks	10.4250	1.15510	283.243	1.851 E-12
	6 weeks	18.1720	.88580		
TiAl ₇ Nb	2 weeks	7.7630	.80089	25.430	8.463 E-5
	6 weeks	9.4200	.66202		
CPTi	2 weeks	3.1440	.77956	177.622	9.157 E-11

6 weeks 11.7840 1.89605

Histomorphometric analysis: Table 4 showed higher Mean values of TB (New bone) of Ti₂AlC in all experimental groups after 2 and 6 weeks implantation in rabbit. A statistical analysis for the comparison among mean values of all experimental group, there was a high statistically significance difference among them with p-value<0.001. Pairwise analysis showed a high statistically

significant association between Ti₂AlC with both CPTi and Ti₆Al₇Nb with p-value<0.001. There was also a high statistically significant difference between CPTi and Ti₆Al₇Nb with p-value<0.001.

Table 4: Bone Tissue (TB) in all experimental groups after 2 and 6 weeks implantation in rabbit.

Time	CPTi	Ti ₆ Al ₇ Nb	Ti ₂ AlC		Mean	SD	F-statistic	P-value
	Mean	SD	Mean	SD				
2weeks	16.060	1.052	17.86	.78294	23.600	.96177	87.906	6.804 E-8
6weeks	27.50	1.000	36.40	.89443	41.600	2.3021	107.415	2.192 E-8
Experimental group	Bonferroni P-value BTT after 2 and 6 weeks							
	2 weeks	6 weeks						
CPTi and Ti ₆ Al ₇ Nb	.031	2.790 E-6						
CPTi and Ti ₂ AlC	7.734 E-8	1.728 E-8						
Ti ₆ Al ₇ Nb and Ti ₂ AlC	1.553 E-6	.001						

Table 5 showed the statistical analysis of data which is represented by the mean values of Bone Implant Contact (BIC), bone tissue surrounding implants after two and six weeks implantation. ANOVA test showed a high statistically significance among all experimental group

after 2 and 6 weeks implantation. Further statistical analysis by using Bonferroni; in the same table P value appear statistically highly significant differences between all pairs of the materials.

Table 5: Bone/implant contact BIC for all experimental groups after 2 and 6 weeks implantation in rabbit.

Time	CPTi	Ti ₆ Al ₇ Nb	Ti ₂ AlC	ANOVA		Mean	SD	F-statistic	P-value
	Mean	SD	Mean	SD					
2 weeks	3.4000	1.140	4.400	1.1401	6.0000	2.0000	3.909	.049	
6 weeks	21.440	1.006	33.56	.72319	38.720	.89833	503.754	2.659E-12	
Experimental group	Bonferroni P-value BIC after 2 and 6 weeks								
	2 weeks	6 weeks							
CPTi and Ti ₆ Al ₇ Nb	.922	1.625 E-10							
CPTi and Ti ₂ AlC	.051	2.472 E-12							
Ti ₆ Al ₇ Nb and Ti ₂ AlC	.341	2.530 E-6							

DISCUSSION

Despite the wide clinical utilization of Titanium implants, there are still potential risks because of the inherent bio inert and easily oxidizable characteristics. For example, the oxide layer of the surface of Titanium often leads to thrombosis between the surface and surrounding tissue, which creates an oral cavity that, promotes microbial reproduction [15]. Moreover, during the operation,

inflammation around the surgical sites may occur due to external heat or pressure. This hinders the normal growth of new bone around the surgical sites and results in weak bonding between the bone and implant [16,17]. MAX phases are a somewhat uncertain kind of material with both metallic and ceramic properties; their classification is still unclear. These carbides possess unusual and even unique physical, chemical, mechanical and electrical properties. They are electrically and

thermally conductive, machinable, and not susceptible to thermal shock, plastic at high temperatures and exceptionally damage tolerant [18-20].

Push out bond strength test had been used to assess the bond strength between implant and bone. Also it is based on shear stress at the interface between implant and bone.

Assessment of Bone-implant interface strength using the pushout test showed a high statistically significant difference between the material used with higher mean values for the strength of the bone-implant interface of the Max phase alloy than both CPTi and Ti₆Al₇Nb at both, 2 weeks and 6 weeks.

The presence of carbon in max phase alloy which appears to stimulate strong cell recruitment during the extensive bone formation which helps in faster healing time, carbon may also cause condensation reactions which provide strong covalent bonds through cell-membrane lipid fatty acids/phosphate/amino-acid end groups, bone phosphate and some organic portions of the bone matrix [21,22].

Osseo integration assessment using the histomorphometric test revealed a high statistically significant difference between the material used with higher mean values for the strength of Osseo integration of the Max phase alloy than both CPTi and Ti₆Al₇Nb at both, 2 weeks and 6 weeks. This could be due to many factors:

Ti₂AlC are exceptionally oxidation resistant which could be because they form a stable and adherent protective Al₂O₃ scale which acts as protective alumina scale [24]. To see how bonding could determine the protective scale formed. The conclusion of Zhou and Sun was that as the bonding created between Ti-C is strongly covalent and the one for Ti-Al is weak. The strength of the covalent bond would decrease the activity of Ti, therefore increasing the activity of Al, which is high enough then to be preferentially oxidized. These two factors mentioned; the low Al content to form a protective scale and the bonding, make the formation of a continuous Al₂O₃ layer on Ti₂AlC favourable. Studies carried out by Meier [25]. Done in Ti-Al alloy showed that they do not create a protective alumina scale but rather a scale composed of TiO₂ and Al₂O₃. Biocompatibility could be mainly due to the excellent corrosion behaviour of the alloy in the physiological environment by a tenacious layer of protective alumina scale or the layer of scale TiO₂ and Al₂O₃ that appear on the implant's surface immediately after exposure to oxygen [26-29].

CONCLUSION

In comparison CpTi has high affinity for oxygen which allows the spontaneous formation, on the Ti surface, of a layer of Ti oxides, mainly TiO₂. Those oxides represent a non-metallic layer on the Ti surface that, in the harsh conditions of biological fluids, has a tendency to grow up, constituting a brittle interface between the implant and the bone.

Ti surface is spontaneously passivated by a layer of oxides, mainly TiO₂, which confer to the surface its high biocompatibility as suggested by many researchers. TiO₂ layer is a non-metallic film interposed between the implant and the bone; it is also very brittle, may be easily fractured, exposing the bulk Ti of the implant to the attack of the harsh conditions of biological environment, with a consequent production of Ti particles. These last might be toxic and induce an inflammatory reaction.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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