Evaluation of Cytotoxicity and Biocompatibility of Ti_2AlC in Rabbits

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ABSTRACT

Background: The Titanium and its alloys are suitable for dental implant and medical applications. Biocompatibility of the materials is a major factor in determining the success of the implant and has a great impact on their rate of osseointegration. The aim of this study was to evaluate the biocompatibility and cytotoxicity of Ti_2AlC in comparison to CPTi & Ti_6Al_7Nb in rabbits.

Materials and Methods: 10 male New Zealand White rabbits, weighing (2-2.5 kg), aged (10-12 months) were used in this study. Cylindrical implants were prepared from the study materials (CPTi, Ti_6Al_7Nb and Ti_2AlC) with (8mm) height and (3mm) diameter for the evaluation of tissue response and disc specimens were prepared with (6 mm) diameter and (2 mm) thickness for evaluation of cytotoxicity MTT test. A histological study was performed at 2 & 6 weeks post- surgical implant insertion.

Results: Histological findings show that Ti_2AlC has enhanced proliferation of osteo-progenitor cell and reported mature bone formation at 6 weeks. Moreover, Ti_2AlC has recorded a higher percentage for viable cells by MTT test in comparison to CPTi and Ti_6Al_7Nb.

Conclusion: The new Ti2AlC dental implant is considered biocompatible and has showed a better bone formation than the CPTi and Ti6Al7Nb materials at 2 & 6 weeks.

Keywords: Bone healing, CPTi, Ti_6Al_7Nb, Ti_2AlC, Osseointegration, Dental implant, . **(Received: 22/9/2021, Accepted: 13/10/2021)**

INTRODUCTION

Titanium regards as a key factor for the establishment of implant tissue interaction and for the assessment of biocompatibility of its alloy [1]. Titanium is applicate in many studies in prosthodontics, conservative and in orthodontics due to their resistance to corrosion and their good tolerance by tissue without causing harms or damage. [2,3,4] Titanium and its alloys may release ions in saliva that contact the oral mucosa and may cause tissue reaction including toxicity or allergy reaction [5,6].

Most researches record that titanium is the least metal material that induces allergy; therefore, it is regarded as material of choice for biological application.

Moreover, Ti_6Al_7Nb alloy is light in weight, have very high tensile strength and well tolerated by bone tissue and reported to be used for biomedical purposes [7,8,9]

The evaluation of cytotoxicity of implant materials along with its osseointegration and bone formation potential becomes important concerning the clinical application of these materials in service and their success in implantation. The relationship between viability of bone cell that contact implant surface and tissue reaction have been recorded in several studies [10,11,12].

The objective of this study was to evaluate the cytotoxicity and bone tissue response in rabbit for the new prepared Ti_2AlC implant in comparison to Commercially pure titanium CPTi and Ti_6Al_7Nb alloy by using histological examination and Methyl thiazolyl- tetrazolium MTT assay at different periods.

MATERIALS AND METHODS

Animals

A total of 10 male New Zealand White rabbits, weighing (2-2.5 kg) and aged (10-12 months) were used in this study, and kept in the animal department of (National Center of Drug Control and Research /Iraq) at a constant humidity and temperature of 23°C according to the National

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Council's guide for the care of laboratory animals.

The following materials were used in this study:

-CPTi rods and Ti_6Al_7Nb rods, 6 mm in diameter from Straumann Company, Switzerland.

-Ti_2AlC powder ASTM E8M03 (Famouschem Technology Shanghai) was used to prepare implant, by using (0.5g) of powder of Ti_2AlC that was condensed by dental condenser of (0.5mm) size. The punch was allowed to seat over the solid steal rod and when the mold was filled with a condensed powder, compaction was started by using a punch guide. Pressing with hydraulic press started using (100 Mpa) for (10min). The specimen was ejected by using the long punch after that the base removed and left for drying 24 hours at room temperature.

Cylindrical implants were prepared from the study materials with (8mm) height and (3mm) diameter for evaluation of tissue response and disc specimens were prepared with (6 mm) diameter and (2 mm) thickness for evaluation of cytotoxicity assessment by MTT test. [13]

In Vivo study

Three implants were implanted in the proximal third of the lateral aspect of the femoral bone, the Ti_2AlC and Ti_6Al_7Nb implant were applicate in the right femur while CPTi was implanted in the left femur. According to the healing interval, the experimental rabbits were divided into two groups (2, 6 weeks), each group consists of 5 animals sacrificed for histological study.

In Vitro Study (Cytotoxicity Test)

Cultured for fibroblast cell line (murine NIH 3T3 Cell Line 93061524 – Sigma) in Dulbecco's Modified Eagle medium. Seed the cells in a 96-well microplate at a density of (1 x 104 with 100 µl) per well. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 in air. In the present study, 6 cut samples from each rod of CPTi, Ti_6Al_7Nb and Ti_2AlC were used for cytotoxicity evaluation with fibroblast cells. Cells were treated with different doses of examined materials. Then, these cells were estimated for their proliferation and viability by Methyl thiazolyl- tetrazolium MTT colorimetric assay, using spectrophotometer record the absorbance at 570 nm as described by Wang *et al*. [14]. Percentage viability was calculated as follows:

$$\text{Percentage Viability} = \frac{\text{absorbance of the test samples} - \text{absorbance of the blank}}{\text{absorbance of control well} - \text{absorbance of the blank}} \times 100$$

Statistical Analysis

All records were entered into Excel spread sheets for evaluation with the Statistical package deal for social studies (SPSS) (Chicago, IL, united states of America). The data were analyzed using one-way ANOVA test.

RESULTS

1.Histological findings: microscopic features for all specimens of implant for **CPTi** group at 2 weeks post-operative duration, show a sparse of bone trabeculae surrounding by osteoblast with basal bone around implant bed. At 6 weeks post-operative duration, the specimens show basal bone coalesce with newly formed thin bone trabeculae at the bed implant region, with presence of fibrous tissue surrounding implant figure 1 (A&B).

Microscopic evaluation for all specimens of implant for **Ti_6Al_7Nb** group at 2 weeks post-operative duration shows bone marrow with a sparse of bone trabeculae coalesce with basal bone, while at 6 weeks post-operative duration, the specimens show a thin rim of fibrous tissue surrounding the implant with bone trabeculae full most of implant bed, figure 1 (C&D).

Implant for **Ti_2AlC** group at 2 weeks post-operative duration shows basal bone with attached newly formed bone trabeculae surrounded by active proliferating osteogenic cells. At 6 weeks all specimens show mature bone surrounding the implant, figure1 (E&F).

2.MTT Results

The results of cytotoxicity of CPTi, Ti_6Al_7Nb and Ti_2AlC by detection and estimation of viable cells for the whole concentration that used for MTT test after 72 h are illustrated in figure (2) and table (1). The material (Ti_2AlC) showed a higher percentage of cell viability (89.6461 ±7.6468) followed by Ti_6Al_7Nb (80.6306 ±5.6362). A significant P value (.001) is recorded for cell viability within and between the examined materials by using ANOVA test, table (2).

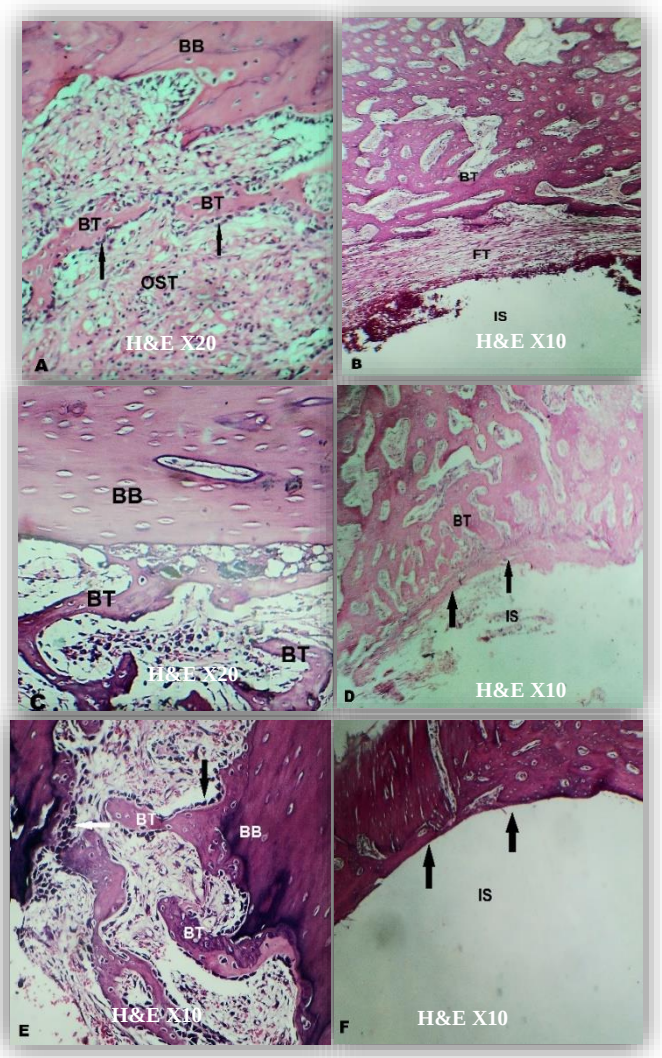

Figure (1) Microscopic view for different examined materials at (2 & 6 weeks) where basal bone (BB), few scattered bone trabeculae (BT), Osteoid tissue (OST), Osteoblast (arrows).
A. CpTi implant at 2-week duration
B. CpTi implant at 6-week duration
C. Ti_6Al_7Nb implant at 2-week duration
D. $Ti6Al_7Nb$ implant at 6week duration
E. Ti_2AlC implant at 2-week duration
F. Ti_2AlC implant at 6- week duration

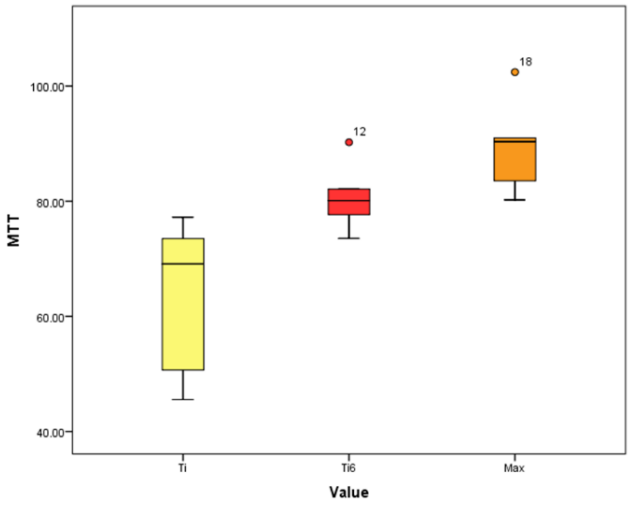

Figure (2) Cell viability of CPT(Ti), Ti_6Al_7Nb (Ti6) and Ti_2AlC(max) after 72 h.

Table (1) Descriptive statistic for MTT assay

Material	N	Mean	Std.	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
CpTi	6	64.19	12.95	50.596	77.796
Ti_6Al_7Nb	6	80.63	5.63	74.715	86.545
Ti_2AlC	6	89.64	7.46	81.621	97.670
Test of homoginity					
Levene Statistic	df1	df2	Sig.		
3.364	2	15	.062		

Table (2) ANOVA Test for the all studied groups for MTT assay

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1998.141	2	999.070	11.609	.001
Within Groups	1290.920	15	86.061		
Total	3289.060	17			

DISCUSSION

Titanium and their alloys implant have been widely used in various branches of dentistry. As implant materials have direct contact with the bone tissue and may interact with cells of the body, therefore, their success not only require an acceptable physical and chemical properties but also must have good biocompatibility [15,16] .In vivo study hasbeen done by implantation of different materials (CpTi, Ti_6Al_7Nb and Ti_2AlC) to investigate their ability in enhancement of osseo-integration and bone formation .Our results for Ti_2AlC implant report an obvious proliferation of osteo- progenitor cells at 2 weeks and a well mature bone formation at 6 weeks in comparison to CpTi, Ti_6Al_7Nb which recorded a rim of fibrous tissue around the implant with bone trabeculae filled more than half of implant bed, Although Ti_6Al_7Nb alloy showed more bone formation than CpTi, immature bone was detected in most of their examined specimens . Many studies revealed that titanium and Ti_6Al_7Nb alloy were used in dental implant due to their excellent compatibility with surrounding tissues [17,18]. On the other hand, the present results focus on excellent findings related to tissue response by newly Ti_2AlC implant material.

In vitro studies have been performed by using of cytotoxicity test to evaluate the biological effects of the examined materials on growth and viability of fibroblast cell which is derived from the mesenchymal layer as having the same origin of the osteoblast cells. The cell viability was recorded by MTT test thatwas based on mitochondrial enzyme which reduced the yellow MTT dye into insoluble Formazan, and the number of viable cells were calculated [19,20,21]. The results indicated that Ti_2AlC material showed a higher percentage of viable cells in whole recorded concentration that coincided and supported the histological findings in better bone formation and maturation in comparison to CPTi and Ti_6Al_7Nb materials.

CONCLUSION

The present study concludes that the new Ti_2AlC implant material is considered a biocompatible and less toxic to cells by recording high percentage of cell viability and showing a better bone formation than the CpTi and Ti_6Al_7Nb materials at 2and 6-week period.

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Conflict of interest: There are no conflicts of interests.

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الخلاصة

الخلفية: يُعَدُّ التيتانيوم وسبائكه مناسباً لزراعة الأسنان والتطبيقات الطبية، إذ يمثّل التوافق الحيوي (البيولوجي) للمواد عاملاً رئيسياً في تحديد نجاح عملية الزرع وله تأثير كبير على معدل اندماجها العظمي. كان الهدف من هذه الدراسة هو تقييم التوافق الحيوي (البيولوجي) والسمية الخلوية لكربيد الألومنيوم التيتانيوم (Ti_2AlC) مقارنة بـالتيتانيوم النقي تجارياً (CPTi) وسبائك التيتانيوم الطبية (Ti_6Al_7Nb) في الأرانب.

مواد البحث وطرقه: تم استخدام 10 من ذكور الأرانب النيوزيلندية البيضاء في هذه الدراسة، بوزن (2-2.5 كغم) لكل منها، وتتراوح أعمارها بين (10-12 شهراً). وتم تحضير زرعات أسطوانية من مواد الدراسة (CPTi، Ti_6Al_7Nb و Ti_2AlC) بارتفاع (8 ملم) وبقطر (3 ملم) لتقييم استجابة الأنسجة، وإعداد عينات قرصية بقطر (6 ملم) وسمك (2 ملم) لتقييم السمية الخلوية عن طريق اختبار MTT. وأجريت الدراسة النسيجية بعد أسبوعين و6 أسابيع من وضع الزرعة بعد الجراحة.

النتائج: تظهر النتائج النسيجية أن الـ Ti_2AlC عزّز من تكاثر الخلايا السلفية (الأولية) العظمية، ولاحظ تكوين عظام ناضجة في غضون 6 أسابيع. علاوة على ذلك، سجل الـ Ti_2AlC نسبة مئوية أعلى للخلايا الحيوية عن طريق اختبار MTT مقارنةً بـالـ CPTi والـ Ti_6Al_7Nb.

الاستنتاجات: تعتبر زرعات الأسنان المحضرة من مادة الـ Ti_2AlC الجديدة متوافقة حيوياً، وأظهرت تكوين أفضل للعظام مقارنة بمواد الـ CPTi والـ Ti_6Al_7Nb خلال أسبوعين و6 أسابيع.

الكلمات الرئيسة: شفاء العظام ، CPTi، Ti_6Al_7Nb، Ti_2AlC، الاندماج العظمي، زراعة الأسنان