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Exploring the Cytotoxicity of Grade IV Commercially Pure Titanium and Grade V (Ti-6Al-4V) Titanium Alloy: Implications for Dental Implant Biocompatibility

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ABSTRACT: The aim of this study was to assess the cytotoxicity between Grade IV CpTi and Grade V Ti-6Al-4V titanium alloy. Both are materials used in dental implants. The effect of these materials on the survival of L929 fibroblast cells, which play a major role in implant integration, was determined using the MTT assay. Cell viability measured at 24, 48 and 72 h of exposure to the materials revealed that grade IV titanium supported higher cell viability than did grade V titanium alloy. Especially the Grade IV titanium showed a significantly better survival as revealed by ten percent higher survival rate over 24 hours compared to that on the Grade V material, and this data suggests that in two candidate materials with biocompatibility their cellular response could be different each other like this for no-down surface became better. Subsequently, based on the juncture of properties and cost, grade IV titanium is considered the first choice for dental implants although V material can be used depending upon circumstances.

KEYWORDS: Cytotoxicity; MTT assay; Ti-6Al-4V; Grade IV titanium alloy.

I. INTRODUCTION

Titanium and its alloys have been the metal of choice for at least dental implants since 1981. This is due to their very good corrosion resistance, high modulus of elasticity and well tolerance behaviour for biological exposure[1]. The success of a dental implant is based on osseointegration as well as the establishment of a soft tissue seal. Gingival fibroblast is very important to preserve connective tissue adhesion and inhibiting epithelial al downgrowth[2].

Commercial pure titanium (cpTi) is divided into four grades, I – IV. “Their strength comes from the greater degree of interstitial,” he said, like iron and oxygen. “Grade IV is the strongest.” For enhanced mechanical strength titanium can be alloyed with aluminium and vanadium to produce Grade V titanium (Ti-6Al-4V). This increased strength is advantageous in the case of narrow or short implants and due to occlusal loading[3].

However, the use of Grade V alloy is known to result in the release of aluminium and vanadium ions that could cause biological concerns. Aluminium, in particular, has been linked to growth sheet-retarding properties in bone, and vanadium is also cytotoxic and can tap allergic reactions[4]. While the amounts released are typically below harmful threshold values, the risk of deterioration and particle release, especially in the context of peri-implantitis or acidic conditions can be critical.

Biocompatibility is a major requirement for every implant material, which is usually evaluated in the first instance according to defined international standards such as ISO 7405 and ISO 10993-5 by means of in vitro tests on cytotoxicity. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) has been widely used for this purpose, and permanent cell lines, including mouse fibroblasts (L-929), are frequently employed to achieve a steady and controllable testing model[5].

Although both Grade IV commercially pure titanium (CpTi) and Grade V Ti-6Al-4V alloy have been widely used for dental implant devices in clinical applications, a quantitative evidence-based analysis of their relative cytotoxic profile and influence on the long-term survival of peri-implant cells is still necessary. To the against discussed deficits, in this investigation that gap was addressed by testing material influence on L929 fibroblast viability to determine the best design properties for maximizing osseointegration[6].Thus, this experiment compared the cytotoxicity of commercialized #Grade-IV pure Ti and its alloy, Ti-6Al-4V (Grade V), on gingival fibroblasts. Cell viability was measured by the MTT assay at 24, 48 and 72

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hours[7]. The aim of this comparison is to supply scientific data regarding cellular response and biocompatibility of these 2 main dental implant materials. The hypothesis is that the cytotoxicity and biocompatibility of Ti-6Al-4V (Grade V) titanium alloy would not differ significantly when compared to Grade IV Commercially Pure Titanium.

II. METHODOLOGY

Sample Description and Preparation:

The disk specimens used (n = 32) were divided into two groups: 16 of pure titanium Grade IV commercially pure (cpTi, ASTM F67; n=16) and 16 of titanium alloy Grade V (Ti-6Al-4V). The disks were 6 mm in diameter and had a thickness of 2 mm for Grade IV and 8 mm in diameter for the Grade V.

All specimens were subject to metallographic grinding with a white coarse grit wheel and finally finished with the white knife-edge rubber aluminum oxide wheel. Thereafter, they were ultrasonically cleaned in deionized water for 10 minutes, re-cleaned in 70% propanol under ultrasound for another 10 minutes to eliminate any impurities. At last all disks were sterilized by autoclaving for 20 minutes at 134°C and 2bar of gauge pressure.

Study Groups and Treatments:

There were two test and two control groups in the experimental design:

Negative control group: Cells incubated with RPMI containing 10% FBS without the treated compounds.

Positivecontrol group: Treated with 10% DMSO (Dimethyl Sulfoxide) and 1% Phenol (cells should show cytotoxic effect).

Test group (CpTi Grade IV): Cells exposed to extracts from the Grade IV disks (n=16).

Test group (Grade V): Cells exposed to extracts of Grade V discs (n=16).

Cell Culture and Extraction:

Mouse fibroblast cells (L929) were grown in RPMI1640 medium supplemented with 10% FBS and 1% antibiotic (penicillin/streptomycin) in a culture plate (24-well). The cultures were incubated at 37°C in 5% CO2 humid atmosphere. When cells were ≧80% confluent, titanium disks were added to the culture medium in accordance with ISO 7405. The cells were incubated for the time periods of 24, 48, and 72 hours.

Cell Viability Assay (MTT Assay):

At 24, 48 and 72 h, the original media was removed and an MTT (5 mg/mL) solution was added. The cells were incubated for 4 h, allowing mitochondrial dehydrogenase activity in viable cells to convert yellow MTT to purple formazan crystals. The MTT solution was then aspirated, and 500 µL of DMSO per well were added to solubilize the crystals. The resulting solution was measured its optical density (OD value) at 570 nm with a UV/VIS spectrophotometer (Multiskan GO, Thermo Fisher Scientific, U.S.A.). Cell viability (%) was normalized and compared to control cells (Figure 1).

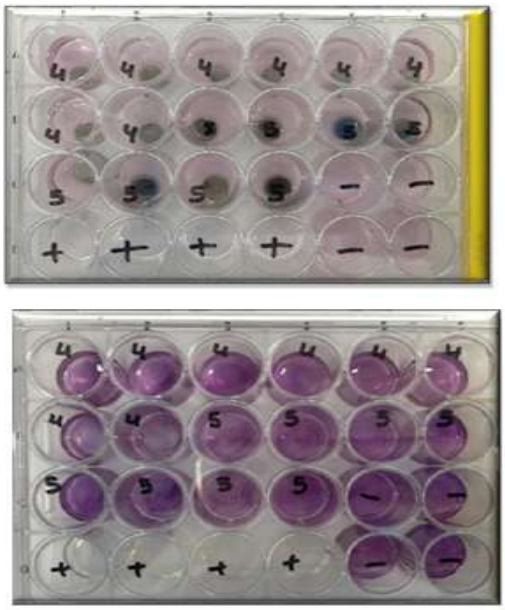

Figure 1. 24-well cell plate after fixation and formazan crystals staining.The color change during formazan crystal staining reflects cell viability: purple indicates the presence of viable, metabolically active cells that have converted MTT to formazan crystals, while transparent signifies non-viable cells or insufficient crystal formation following dissolution. The intensity of the purple color correlates with the number of living cells, providing insight into their metabolic state.

Data analysis:

Statistical analysis was done by SPSS package, version 26. The data distribution was also normal by the Shapiro-Wilk's test ($p>0.05$). A T-test for independent samples was applied to measure cell viability in both titanium grades at all the time intervals. Time effect on cell viability within each grade was analyzed by one-way repeated measures ANOVA and subsequent post-hoc Tukey's test. $P<0.05$ was considered as a threshold for statistical significance.

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III. RESULTS

Cell viability results are presented in Figure 2and Table 1. The cell viability between Ti alloy (grade IV) and Ti (grade V) was significantly different. At 24 h, a 10% increased cell viability was observed on Grade IV Ti (80.3 ± 5.1%) relative to Grade V titanium alloy (70.3 ± 4.1%), and this trend persisted at the 48 h and 72 h time-points. Cell viability of Grade IV titanium at 48 hours rose to 85.2 ± 3.9% from 80.3 ± 4.5% for Grade V ($p < 0.001$). Similarly, at 72 h, commercial pure titanium demonstrated higher performance than Titanium alloy, with a cell viability of 87.1 ± 3.2 and 81.7 ± 4.2% respectively ($p < 0,001$). The presented data indicate that while GIV Ti supports high levels of Fb viability over time, GV does so to a lesser extent.

The one-way repeated measures ANOVA and post hoc analysis results showed a significant time effect on cell viability for both titanium materials (Grade IV: p=0.001; Grade V: p=0.001). Grade IV and V: For both Grade IV and Grade V, the post-hoc analysis revealed that there is a significant difference in cell viability between 24 h and 48 h (Grade IV: p=0.338; Grade V: p=0.272). Both groups have a significant difference in viability at 72 hours when compared to 24 and 48 hours ($p<0.003$). This increase was more pronounced in Grade V (24 hours to 72 hours increased by 11.4%) than in Grade IV (24 hours to 72 hours), which was increased by 6.9%. These findings suggest that although Grade IV cells began with the greater viability, both groups appear to have increase in cell survival over 72 hours.

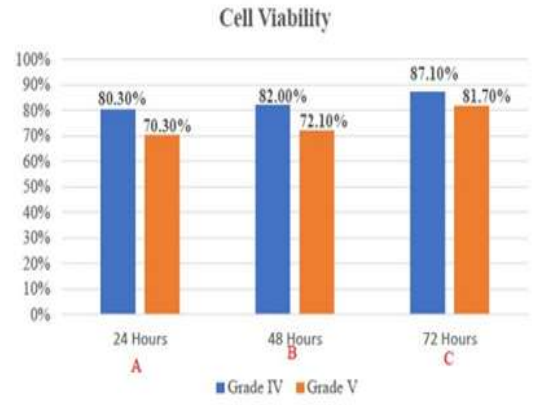

Figure 2. Diagram describing the cell viability among the groups at 24 hr,48 hr and 72 hr of medium extracts of titanium alloys obtained from MTT assay.

Table 1: Cell viability mean and standard deviation values of different tested groupsamong time intervals.

Time Interval	Grade IV	Grade V	p-Value
24 Hours	80.3pm5.1	70.3pm4.1	0.001
48 Hours	82.0pm6.7	72.1pm3.2	0.001
72 Hours	87.1pm2.5	81.7pm4.4	0.001

IV. DISCUSSION

The aim of this study was to evaluate the cytotoxicity of commercially pure (CpTi) Grade IV titanium and Ti-6Al-4V Grade V titanium alloy on L929 fibroblasts, one of the main cellular components taking part in the healing process around a dental implant. This was accomplished through in vitro application and evaluation of cell viability after 24, 48, and 72 hours using the [3-(4,5di-methylthiazol-2-yl)-2,5diphenyltetrazoliumbromide] (MTT) assay[8]. Biocompatibility of both materials was good but cytotoxicity assays led to significant differences between both materials, with Grade IV titanium promoting consistently better cell viability than Grade V titanium alloy[9].

Results from the MTT assay demonstrated that fibroblasts were more viable on Grade IV titanium in comparison to Grade V titanium alloy at all time points tested. In the24-hour period, Grade IV Ti recorded a 10% greater cell viability (80.3 ± 5.1%) compared to Grade V Ti (70.3 ± 4.1%), and this difference increased with time at 48 and 72 hours. One potential explanation for this higher cell survival in Grade IV titanium would be the lack of potentially toxic elements like aluminum and vanadium that are found in Grade V titanium alloys. Aluminum and vanadium have also been reported to cause adverse biological reactions such as cytotoxicity and allergy at high concentrations. For example, vanadium has been demonstrated to disrupt cell metabolism and induce cellular stress and inflammation that may affect the long-term success of implants[10].

One important factor influencing the elevated cytotoxicity response in Grade V Ti alloy might be the liberation of metal ions from this alloy into tissues. In particular, vanadium has been recognized as a possible trigger of allergies and toxicity, which may slow down recovery around the dental implant. Although the content of these elements in the alloy is much lower than that required to cause severe toxicity, low amounts are sufficient to participate in inflammatory reactions and retard wound healing. On the other hand, Grade IV titanium is purer and does not contain such elements as Ni and Al, so it can show a

positive role for cellular response compared to that in this study[11].

Mechanical characteristics of the two materials are another consideration. A commonly preferred alloy is Ti-6Al-4V (Grade V) due to its high strength and stiffness in comparison to other grades making it fit for heavy load carrying applications. Yet, these mechanical benefits are counterbalanced by a lack of biocompatibility. Commercially pure titanium (Ti), such as Grade IV Ti, is not as strong as Ti-6Al-4V; however, it maintains an acceptable degree of strength for dental implants and has a better strength versus cytocompatibility ratio. This render Grade IV titanium an excellent material for uses where tissue healing and integration are the primary concerns, such as in dental implants, which require long-term stability and soft-tissue compatibility[12].

Similarly, the cell viability of the two materials was increased at all time points and to a greater extend between 24 hours after transplantation until 48 hours. This means that both Ti grade IV and V alloy have the potential to stimulate cell proliferation during early healing phase, helping the implant to integrate. Nonetheless, in general Grade IV titanium system offers a more predictable favorable place for cell survival and tissue regeneration. This is in keeping with the findings indicating a direct relationship between cell viability and the absence of toxic metal ions and good surface interaction between titanium and fibroblasts[13].

These finding are in agreement with those of many published reports on the cytotoxicity of titanium and its alloys. For instance, it has been reported that titanium in particular as commercially pure can be considered well-tolerable within the human organism or only cause slight cellular reactions. On the other hand, titanium based Ti6Al4V alloys presented variable results but are still biocompatible due to the presence of aluminum and vanadium. It has been suggested that these metals can contribute to delayed healing and inflammation hence affecting the success of dental implants[14]. Our findings are in support of these observations that Grade IV titanium was favorable for survival of fibroblasts therefore would provide a better long-term result in tissue healing and integration[15,16].

There are a number of limitations to this research as an in vitro study. It is unable to reproduce the complicated in vivo environment, host immunologic responses, blood perfusion, bacteria biofilm formation, biomechanical loading and three-dimensional structure of soft tissue seal. Although L929 fibroblasts are a good cell line for cytotoxicity screening, their response may not be fully representative of that of human gingival fibroblasts, and other important cells such as osteoblasts and keratinocytes in situ.

Nevertheless, it is interesting to know that Grade IV seems more slightly biocompatible at a cellular level, which could be used as reference for future in vivo analysis. It supports the concern of the alloying elements in Grade V, which can potentially become more prone to localized problems such as peri-implantitis from particle release secondary to tribo-corrosion.

Additional comparisons in particular longer-term in vivo studies, are needed to more fully evaluate the long-term biologic response and clinical outcome of these two titanium grades

V. CONCLUSION

In summary, these in vitro results suggest that both Grade IV commercially pure titanium and Grade V Ti-6Al-4V titanium alloy has reasonable biocompatibility rate for the culture with human gingival fibroblasts. Nonetheless, for dental implants, which require long-term compatibility with the tissue implanted, these results confirm Grade IV titanium as a more suitable implant material to ensure patient satisfaction and compatibility over an extended period in comparison to Grade V alloy. Although the Ti-6Al-4V alloy is a possible material for dental implants due to its mechanical properties, it would be important that future studies improve the composition and surface treatment of these implants in order to reduce their cytotoxic effect. The results of this study strongly suggested that Grade IV titanium should be used in clinical situations where biocompatibility and soft tissue healing are deemed important, thereby supporting concise decision-making in dental implantology

Abbreviations

MTT:(3-(4,5-Dimethylthiazolyl-2)-2,5diphenyltetrazolium bromide
CpTi: Commercial Pure Titanium
Ti6Al4V: 90% Ti, 6% Al, 4% V
OD:optical density
DMSO: Dimethyl Sulfoxide
FBS: Fetal Bovine Serum

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