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Biocompatible and mechanical properties of low temperature deposited quaternary (Ti, Al,V) N coatings on Ti6Al4V titanium alloy substrates

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A series of quaternary (Ti, Al, V) N coating layers were obtained by low temperature reactive plasma sputtering in differing deposition conditions to improve the wear resistance and the biocompatibility of a titanium surgical alloy, specifically Ti-6Al-4V. Characterization of the mechanical properties, structure and the chemical composition of the coating layer was explored by microhardness test, ball against flat wear test, scanning electron microscopy and X-ray diffraction. The biocompatibility of the optimum coating layer (as determined by mechanical performance) was examined by a modified MTT toxicity test and by monitoring cell growth assessed by quantitative stereological analysis. The experimental results are encouraging, indicating that this low temperature deposited, dense, quaternary (Ti, Al,V) N coating layer exhibits improved mechanical properties such as high hardness and excellent adhesion to a Ti alloy substrate and is highly biocompatible.

1. Introduction

Ti6A14V alloys have been widely used in orthopaedic applications such as dental implants, artificial joints, and bone plates. This alloy tends to be chosen due to its commercial popularity and availability although there are other titanium alloys which demonstrate similar or even improved properties such as corrosion and fatigue resistance, and biocompatibility.

Studies on titanium alloy implants have shown that metallic ions are released from the alloys [1–3]. These ions, even at low concentrations, may cause local irritation of the tissue surrounding the implant and in the long term the patient may become sensitized to the released metallic ions. The presence of particulate titanium alloy debris, macrophages and T-lymphocytes in the fibrous tissue around both failed cemented and cementless replacement hip and knee joints indicates that the outcome of the interaction between wear particles and cells in the implant–tissue interface may be crucial for the long-term stability and function of the prosthesis [4–6].

Several surface modification techniques such as ion implantation, high temperature nitriding and chemical passivation have been used to create a nitride or oxide layer onto titanium alloys to increase the tribological behaviour of the articular prostheses [7–11]. However, such coating layers normally require high temperatures to produce optimum structural and mechanical properties. These elevated temperatures may be sufficiently high to detrimentally alter the microstructure and fatigue properties of the titanium alloy substrates used in the prosthesis.

In this study a quaternary (Ti, Al,V) N coating layer has been developed as an alternative to the TiN coatings of the last decade. It has many advantages such as high hardness, low coating temperature, and excellent adhesion strength to the substrate. A quaternary (Ti, Al,V) N coating has been shown to significantly improve the wear and friction properties of titanium alloys and high speed stainless steel tools [12, 13]. However, before quaternary (Ti, Al,V) N coatings are introduced as a potential biomaterial, the biocompatibility of such coating layers must be evaluated.

Therefore, the objectives of this study were to prepare and assess the mechanical properties of candidate compositions of (Ti, Al,V) N coating layers, and evaluate the biocompatibility of an optimized coating using an *in vitro* toxicity test as a first stage, followed by a preliminary quantitative cytocompatibility study for cell growth.

2. Materials and methods

2.1. Ti-alloy samples

The titanium alloy was commercially obtained in its annealed condition with a composition 6.48% Al, 4.13%V and titanium as balance. Two geometries of sample were used for the coating evaluation and biocompatibility tests. The geometry used for the coating evaluation was a rectangular coupon of dimensions $10 \times 15 \times 0.2$ mm. The biocompatibility test samples were punched from 0.2 mm sheet into discs of 15 mm diameter. Both geometries were sanded on SiC paper (grade from 240 to 4000) and finished by polishing with diamond powder of 1 μm.

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TABLE I Processing variables used for the deposition of (Ti, Al,V) N coatings by capacitively coupled RF plasma reactive sputtering apparatus. Series 1 represents the investigation into the effect of chamber pressure while series 2 investigates the effect of nitrogen/argon ratio

Reactive sputtering	Series 1	Series 2
Substrate temperature (°C)	265 ± 15	265 ± 15
Target substrate distance (cm)	5.0 ± 0.2	5.0 ± 0.2
Power density (W cm^{-2})	3.0 ± 0.1	3.0 ± 0.1
Target self bias (V)	− 270 ± 10	−270 ± 10
Nitrogen/Argon (N_2/Ar) ratio ± 0.1%	6%	2%, 4%, 6%, 8%, 10%, 12%, 14%
Chamber pressure (1.33×10^{-1} Pa)	2, 4, 6, 8, 10, 12, 14, 16, 18, 20	4
Sputtering time (h)	8.0 ± 0.1	8.0 ± 0.1

2.2. Reactive sputtering

The range of stoichiometries and crystal structures of the (Ti, Al,V) N coatings were produced by a capacitively coupled RF plasma reactive sputtering apparatus. The sputtering target was Ti–6Al–4V alloy, nitrogen was the reactive gas which was mixed with argon as the working gas. The nitrogen/argon (N_2/Ar) ratio was controlled by two 100 sccm mass flow controllers accurate to ± 0.1% of fsd. The continuously pumped reaction chamber pressure was maintained by feedback from a capacitance manometer fed to the mass flow controllers via a ratio controller. The polished Ti-alloy samples were ultrasonically cleaned in absolute ethanol for 30 min and then *in situ* sputter-cleaned in the reaction chamber with argon as the source gas for 5 min prior to deposition. The pressure and the N_2/Ar ratio were the chosen variables during the reactive sputtering process. All other parameters such as power density, self-bias and temperature were fixed at settings determined in a preliminary study. The reactive sputtering parameters are listed in Table I showing the range of pressures and N_2/Ar ratios investigated.

2.3. Coating evaluation

The coating layers, thickness ~ 5 μm, were analysed by X-ray diffraction (XRD) analysis using CuK_α radiation, scanning electron microscopy (SEM) and Knoop microhardness test. The wear test comprised of a mechanically driven rider, tungsten carbide 5 mm ball, against the coated discs with normal load 14 N. The drive force was monitored by means of a strain gauge arm. The amplitude of the oscillation was 100 μm peak-to-peak at a frequency of 22 Hz.

2.3.1. MTT cytotoxicity test

For the purpose of this study a murine fibroblast cell line (3T3-L1) was used originally obtained from the European Collection of Animal Cell Cultures. The cells were cultured in Dulbecco's Minimum Essential Medium supplemented with 10% newborn calf serum, in a humidified 5%CO_2 incubator. Alliquots of 2 ml of cell suspension containing 2.5×10^4 cells/ml were transferred into three 24-well culture plates containing triplicate samples of the (Ti, Al,V) N coated Ti alloy. Further alliquots were added to sample-free wells in each plate as controls (media only was added to "blank" wells). After 24 h incubation, culture medium containing sodium dodecyl sulphate (SDS) in concentrations of 50, 100, 150 and 200 μg/ml was added to triplicate wells as a positive control.

After a further 24 h, the wells in one plate were replaced with media containing 100 μg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and incubated for a further 4 h [16]. The MTT solution was then replaced with 0.4 ml/l 1 M HCl in dimethyl sulphoxide (DMSO) and the plate placed on a Rotatest shaker for 10 min to produce a homogeneous coloured solution. The absorbence was then read at wavelength 550 nm (620 nm reference) on an Anthos 2001 plate reader against the "blank" wells containing no cells. This was repeated with the second and third plates after 48 and 72 h, respectively.

2.3.2. Cell morphology by SEM and quantitative analysis

Aliquots of 2 ml of cell suspension containing 2.5 × 10^4 cells/ml were transferred to the wells of a 24-well culture plate containing (Ti, Al,V) N coated and uncoated Ti alloy samples. Culture wells free of samples were used for the negative control surfaces. Following 72 h incubation, the cells were washed free of culture media with prewarmed phosphate buffered saline (PBS) and then fixed with 2.5% gluteraldehyde in 0.1 M cacodylate buffer pH 7.2 for 1 h. The samples were then post-fixed with 1% osmium tetroxide, dehydrated though increasing concentrations of ethanol, critical point dried and gold sputter-coated for the SEM.

Stereological analysis was carried out on scanning electron micrographs of cells grown on the uncoated Ti alloy (denoted surface type B) and the (Ti, Al,V) N coated Ti alloy (denoted surface type D) using the point counting method with a simple square lattice [17]. Briefly, the following estimations were carried out: (i) the relative surface area of the cells S such that $S = A_c/A_s$ where A_c is the area of cell coverage in a randomly sampled area A_s, (ii) the number of cells in the sampled areas N, and (iii) the index of mean cell size I calculated by $I = S/N$ [18].

3. Results

3.1. Deposition and coating microhardness and wear

The effect of changing the deposition partial pressure on the hardness of the deposited film was examined first at a constant N_2/Ar ratio of 6%. The results showed two regimes of Knoop hardness values: (i) 1.3 Pa and above, the coating hardness values were less than $HK_{0.05}1500$; and (ii) less than 1.3 Pa, hardness values were in excess of $HK_{0.05}1500$ with optimum pressure range in the region of 0.3–0.8 Pa.Thus a constant pressure of 0.5 Pa was chosen to examine the variation of N_2/Ar gas ratio with hardness

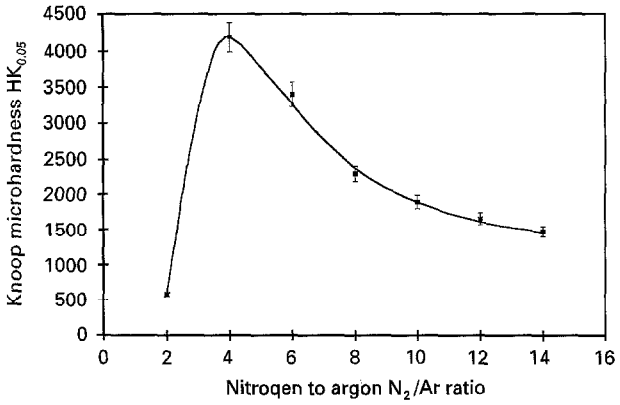

Figure 1 The variation of Knoop microhardness of the (Ti, Al,V) N coating with the processing variable of nitrogen to argon N_2/Ar gas ratio. Chamber pressure was 0.5 Pa.

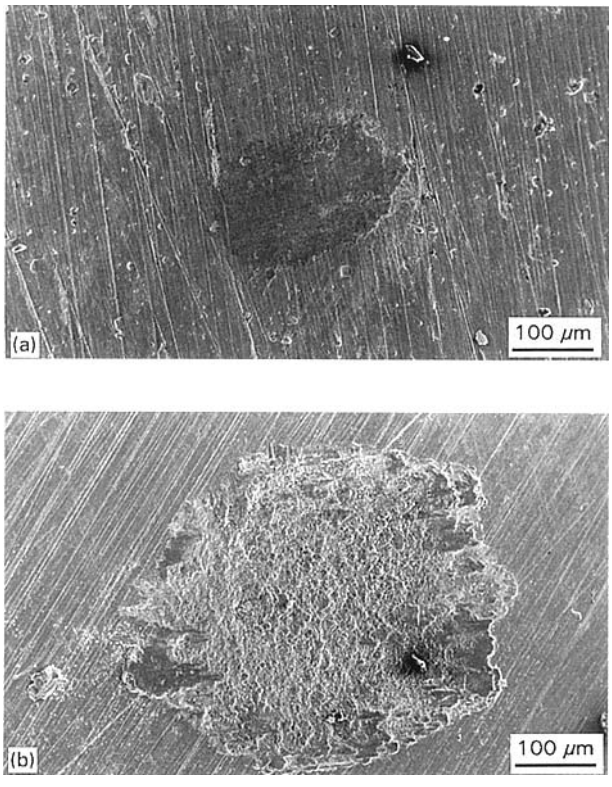

Figure 2 SEM micrographs of (a) the wear test scar on the (Ti, Al,V) N coating produced with an N_2/Ar gas ratio of 4% compared with (b) an uncoated Ti6Al4V alloy sample after 2000 oscillations.

of the (Ti, Al,V) N coating. The results of this are shown in Fig. 1. The microhardness was observed to increase dramatically from $HK_{0.05}$ 580 ± 25 for a N_2/Ar ratio of 2% to a maximum value of $HK_{0.05}$ 4200 ± 250 at 4% N_2/Ar ratio and then decreases gradually as the N_2/Ar ratio increased towards 14% showing an asymptotic tendency to $HK_{0.05}$ 1500. The Ti-6A1-4V alloy without coating was found to have a hardness of 384 ± 10 $HK_{0.05}$.

The SEM micrographs in Fig. 2 show the wear test result on the optimum (Ti, Al,V) N coating layer and the uncoated substrate after 2000 oscillations. The wear scar illustrates that this coating can significantly increase the wear resistance of titanium alloy and shows no observable sign of delamination.

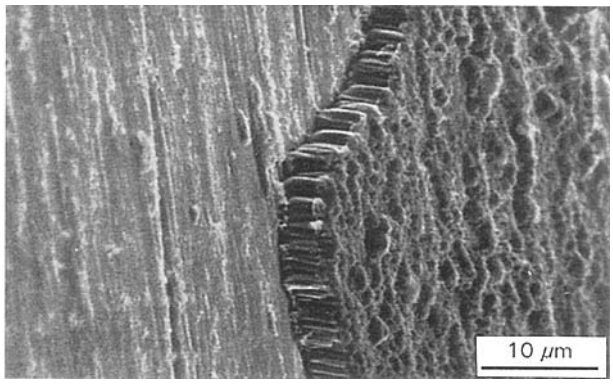

Figure 3 SEM micrograph of the cross-section of the quaternary (Ti, Al,V) N layer for processing parameter of 4% N_2/Ar ratio. The SEM stage was rotated 60° from the normal to the beam.

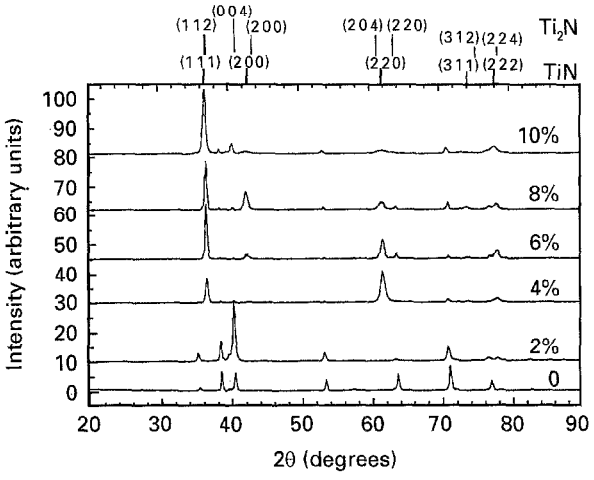

Figure 4 X-ray diffraction spectra for (Ti, Al,V) N coatings for a range of N_2/Ar ratios. Each spectra is labelled on the right hand side 2%, 4%, 6%, 8%, 10% referring to the N_2/Ar ratio in the deposition process. The spectra labelled 0 is the pattern for the uncoated Ti-alloy sample. For comparison the prominent peak positions for TiN and Ti_2N, from the JCPDS-ICDD data file, are positioned along the top of the figure.

3.2. SEM examination and XRD analysis

The (Ti, Al,V) N coatings produced by different deposition parameters on the polished titanium alloy substrates were found to be dense, uniform, and strongly adherent. SEM micrographs of the cross-section of the quaternary (Ti, Al,V) N coating layer showed a very dense structure with a zone II character with respect to the Thornton diagram [14] for deposition substrate temperatures as low as 280 °C, an example of the coating structure is shown in Fig. 3. The X-ray diffraction results of the (Ti, Al,V) N coatings for the range of deposition parameters of N_2/Ar ratios are presented in Fig. 4. The patterns for the 12% and 14% results are similar to the 10% pattern and are not presented on Fig. 4, for the purposes of clarity. The pattern for the uncoated Ti-alloy is included on Fig. 4, represented by 0. There are clear differences in structure with the variation of the N_2/Ar ratio. A detailed interpretation of the diffraction data is to be reported elsewhere [15], however the significant peaks for TiN and Ti_2N are shown on a bar on Fig. 4 so that structural and stoichiometric changes may be discussed here.

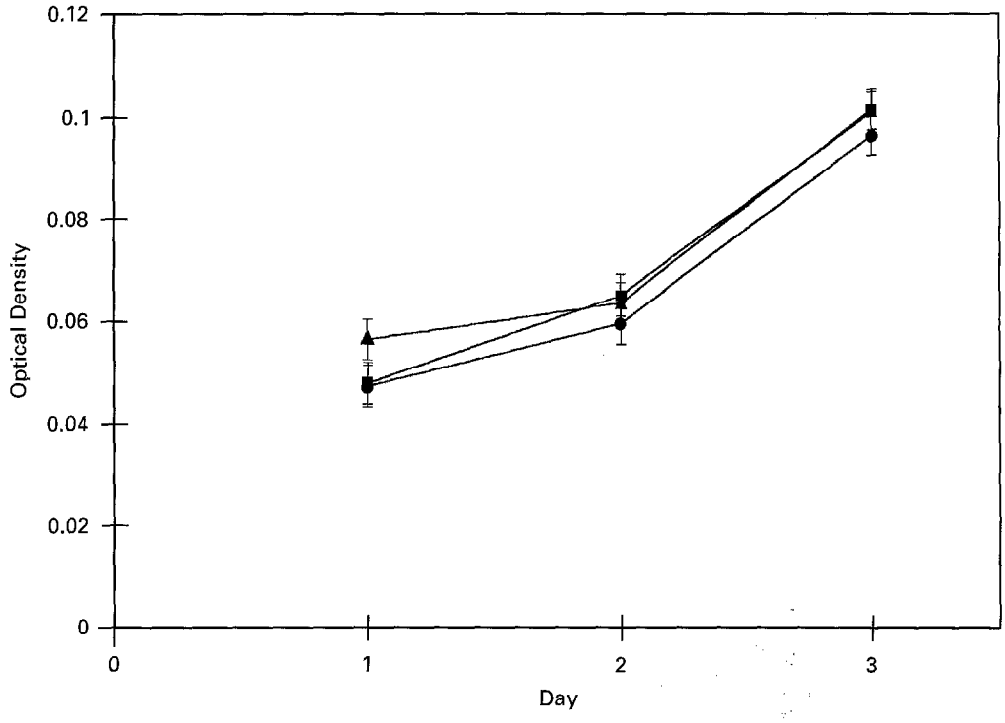

Figure 5 Results of the MTT test for the uncoated and the (Ti, Al,V) N coating with depositions conditions of 4% N_2/Ar ratio. Cells were cultured on samples over a 3-day period. —●— uncoated Ti-alloy, —■— nitride coating, —▲— negative control.

3.2.1. MTT cytotoxicity test

The optimum (Ti, Al, V) N coating (as defined by maximum hardness) was used for the MTT test and compared with samples of uncoated Ti alloy. The effect on the biocompatibility of the other coating conditions and therefore coating composition and structure forms part of a larger study still under investigation.

The results of the MTT test of cells cultured on samples over a three day period are shown in Fig. 5. The level of mitochondrial activity is indicated by the optical density of the reduced MTT. It can be seen that the cells grown on the polyethylene culture plate (negative control), the 4% (Ti, Al,V) N coated Ti-alloy, and the uncoated Ti-alloy samples all exhibit a similar pattern of viability and growth over the 3-day period, with no significant difference between: the control and the (Ti, Al,V) N coated Ti-alloy ($p = 0.78$), the control and the uncoated Ti-alloy ($p = 0.91$), and the uncoated Ti-alloy and (Ti, Al, V) N coated Ti alloy samples ($p = 0.88$). The cells grown on the nitride-coated samples did show a slight increase in viability compared with those on the uncoated Ti-alloy samples, although the difference was not statistically significant.

There is, as expected, a significant difference between the negative control cultures and the positive control cultures to which 100 mg/ml SDS had been added ($p = 0.008$).

3.2.2. Cell morphology

The scanning electron micrographs, Fig. 6, of cells grown on both the uncoated Ti alloy (Fig. 6a) and the (Ti, Al,V) N coated Ti alloy (Fig. 6b) after 2 days show cells exhibiting normal morphological characteristics, with spreading and process formation indicative of viable cell attachment and growth.

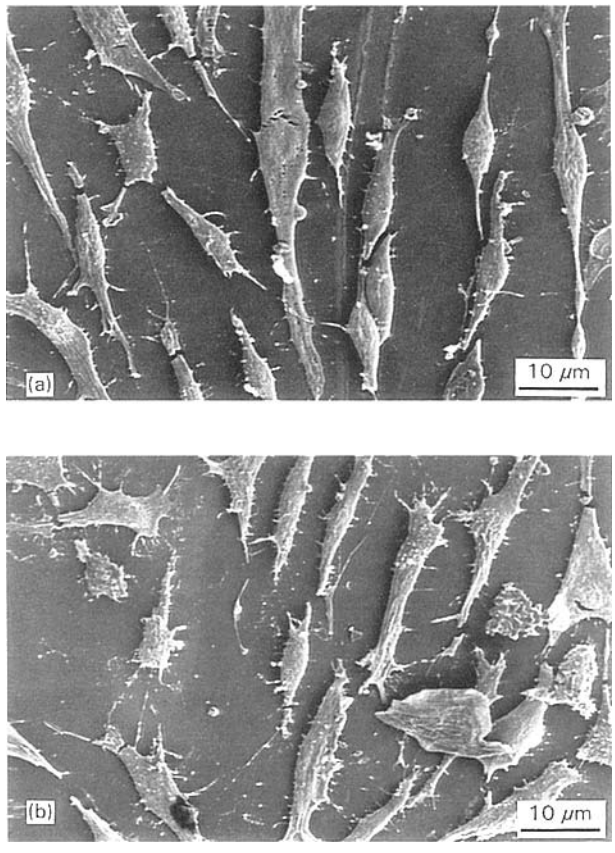

Figure 6 Scanning electron micrographs of cells grown on both the uncoated Ti alloy (a) and the (Ti, Al,V) N coated Ti alloy (b) after 2 days show cells exhibiting normal morphological characteristics, with spreading and process formation indicative of viable cell attachment and growth.

The results of stereological analysis of the number of cells present in the sampled areas (N), and the area covered by cells (S) is shown in Table II. The greater number of cells observed on the (Ti, Al,V) N coated Ti

TABLE II Relative surface area, mean number and index of cell size of cells for the uncoated Ti alloy and the (Ti, Al,V) N coated Ti alloy

	Uncoated Ti alloy	(Ti, Al, V) N coated Ti alloy
Relative surface area covered by cells (S)	0.190 ± 0.026	0.257 ± 0.029
Mean number of cells in sampled areas (N)	13.3 ± 0.7	17.0 ± 0.6
Index of cell size (I)	1.44 ± 0.12	1.51 ± 0.11

alloy as compared with the uncoated Ti alloy would suggest that the initial attachment of cells is higher on the (Ti, Al,V) N coating than on the uncoated Ti alloy. However, the growth rate and degree of spreading following initial attachment is the same for both samples, as shown by the good agreement of the index of cell size. This would indicate, that there is no significant difference in the cytocompatibility between the two substrates, but suggests that the surface characteristics of the (Ti, Al,V) N coated Ti alloy is more conducive to initial cell attachment.

4. Discussion

4.1. Coating microstructure

The X-ray patterns of the coatings produced by the deposition conditions of 2% of N_2/Ar ratio shows a strong zeta-Ti_3N_{2-x} peak along with similarities to the X-ray patterns of the Ti6A14V substrate, denoted 0. However, at 4% N_2/Ar ratio the composition of the coatings is predominantly TiN(2 2 0) and TiN(1 1 1). Evidence of crystal orientation change is observed with increasing N_2/Ar ratio up to 8% where the TiN (2 2 0) peak diminishes and a strong TiN (2 0 0) peak is observed which subsequently diminishes at 10% N_2/Ar ratio where the TiN(2 2 2) peak is evident. Although the Ti_2N(1 1 2) peak is of similar 2θ angle to TiN(1 1 1) there appears little evidence of stoichiometric change from the other peaks of the spectra.

Interestingly the X-ray pattern observed for coating conditions of N_2/Ar ratio of 10% and above is similar to that observed on the same titanium alloy which is nitrided in 950 °C by an ion implantation technique. This and the detail of vanadium and aluminium nitride and other stoichiometric and orientational changes associated with the smaller peaks is to be reported elsewhere [15].

The dramatic increase in hardness may be due to the lattice parameters of (Ti, Al,V) N are only slightly different from the TiN monocell. The smaller Al atoms may replace the Ti atoms in the lattice leading to a shrinkage in the lattice parameters to 0.420 nm as compared to 0.424–0.426 nm for TiN to create a denser structure [14]. This structure has excellent adherence as well as exceptional hardness, showing no sign of delamination even when a coating edge is ground and polished.

The results of the MTT test indicate that cell metabolism, as indexed by mitochondrial activity, is not affected by the (Ti, Al,V) N coating. Moreover, there is a gradual increase in mitochondrial activity over the 72 h period commensurate with sustained cell growth and viability.

The 3T3 cells used in this study are a substrate dependent fibroblastic cell line and consequently obey the following surface interactive sequence: attachment, adhesion, spreading and cell division. Attachment and spreading were specifically monitored in the cytocompatibility assay using an unbiased stereological quantitative method. The fact that cells grown on the (Ti, Al,V) N coating exhibited similar morphology and index of spread with respect to the cells grown on uncoated Ti alloy and that there appeared to be a slight increase in the number of cells attaching to the (Ti, Al,V) N layer strongly indicates that it provides a highly suitable surface for cell growth. Indeed the increase in the number of attaching cells may have been responsible for the slightly higher levels of mitochondrial activity recorded in the (Ti, Al,V) N coated samples. Therefore, these preliminary biocompatibility results indicate that the surface treatment of the Ti alloy resulting in improved mechanical properties did not adversely affect the normal pattern of cell behaviour in culture.

5. Conclusion

The results indicate that (Ti, Al, V) N coating layer may satisfy the critical requirements of bioceramic coating: that of biocompatibility, strong adherence, high hardness and excellent wear resistance. The results of this study are extremely encouraging for the use of (Ti, Al, V) N as a wear resistant coating in joint replacement. The (Ti, Al, V) N coating layer has a similar structure to the TiN coatings and it is unlikely to delaminate up to temperature of 650 °C. It has the advantages of a low temperature process and its dense structure has the potential to be an effective barrier to metal ion release from the prosthesis.

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