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Bone response to a titanium aluminium nitride coating on metallic implants

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Abstract The design, surface characteristics and strength of metallic implants are dependant on their intended use and clinical application. Surface modifications of materials may enable reduction of the time taken for osseointegration and improve the biological response of bio-mechanically favourable metals and alloys. The influence of a titanium aluminium nitride (TAN) coating on the response of bone to commercially pure titanium and austenitic 18/8 stainless steel wire is reported. TAN coated and plain rods of stainless steel and commercially pure titanium were implanted into the mid-shaft of the femur of Wistar rats. The femurs were harvested at four weeks and processed for scanning electron and light microscopy. All implants exhibited a favourable response in bone with no evidence of fibrous encapsulation. There was no significant difference in the amount of new bone formed around the different rods (osseoconduction), however, there was a greater degree of shrinkage separation of bone from the coated rods than from the plain rods ($p = 0.017$ stainless steel and $p = 0.0085$ titanium). TAN coating may result in reduced osseointegration between bone and implant.

1. Introduction

Metal implants are in widespread clinical use; some are intended for short term use, for example; bone pins and cover screws for dental implants, whist others are intended for long term or permanent placement, for example dental implants and orthopaedic joint prostheses. Host response to an implanted device will depend on many factors including the material, design, and surface characteristics of the implant; patient factors such as bone quality and medical status and the surgical technique employed [1, 2, 3, 4,]. Implants intended for long term use should ideally integrate with bone, should not excite an inflammatory response or become surrounded by a fibrous capsule [5]. Materials eliciting the best biological responses may not have the most appropriate mechanical properties and those with good mechanical properties may promote unfavourable biological responses. Various surface treatments and coatings are applied to metal implants to improve tribological properties and to improve bone response [6, 7, 8, 9, 10].

A clinical problem sometimes encountered with titanium dental and extra-oral implants and their abutments is that they are relatively soft and easy to damage. The addition of a hard coating to the surface of titanium or other metallic implants might overcome this problem. However, it is clear that any improvement in durability should not be at the expense of a favourable biological response.

The addition of thin hard coatings to cutting tools has been shown to improve performance and longevity [11, 12]. These coatings are very thin, hard with a low coefficient of friction and may be suitable as coatings for endosseous implants and trans-mucosal or trans-dermal abutments. Coatings for implants should be very thin, strongly adherent to the bulk metal, scratch resistant, not affected by the heat of an autoclave, biocompatible and capable of being applied in a consistent, thin and uniform layer. Physical vapour deposition of titanium aluminium nitride (TAN) onto stainless steel is likely to fulfil most of these requirements [11]

The aim of this study was to assess the bone response to a TAN coating on stainless steel and commercially pure

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titanium and to compare this with the response to the uncoated metals.

2. Materials and methods

2.1. Implants

The experimental implants comprised 18:8 stainless steel orthodontic wire (K.C. Smith and Co., Gwent, UK), coated with titanium aluminium nitride (TAN SS) or left uncoated (SS) and of coated and uncoated commercially pure titanium wire (Goodfellow Cambridge Ltd., Cambridge, UK), (TAN cpTi and cpTi respectively). The wires were 1mm diameter and cut into 2mm lengths with a diamond disc, coatings were therefore present only along the length, and not across the cut ends, of the implants.

Prior to implantation rods were cleaned by phosphate free detergent (Neutrocon, Decon Laboratories Ltd., Hove, East Sussex, UK) in an ultrasonic bath followed by two ten minute rinses in butanol and three ten minute rinses in absolute alcohol, specimens were then allowed to dry in air.

2.2. Coating

TAN coatings were applied to the wires in a splutter coating machine (HTC 1000-4ABS^{TM} Hauzer Techno Coating Europe BV, Netherlands). Wires were cleaned, and then chromium metal ion etched using steered cathodic arc discharge. A 3 μm splutter coating of titanium aluminium nitride (TAN) was applied over 4 hours at 450°C in a vacuum of 6×10^{-4} m.bar; arc current was 100A wire bias voltage of 1200V.

The TAN coating used has been characterised on stainless steel and has the following characteristics:

Young's modulus, 410 Gpa; Surface roughness (Ra), 0.04μm; Hardness (HK), 2500; Co-efficient of friction, 0.6 against Al_2O_3; Stress (compressive), 3.8 Gpa; Crystal orientation, <111> columnar morphology; oxidation resistant up to 850ºC; scratch adhesion, 55N on hard stainless steel and 110N on tungsten carbide [13].

2.3. Surgery

Twenty-eight weaned female Wistar rats were used in this study. Animals were housed in groups in conventional laboratory conditions and provided with standard laboratory food and water *ad libitum.*

The animals were divided into four groups of seven, with each group assigned to one of four test materials, TAN SS, SS, TAN cpTi or cpTi.

One test rod was implanted into the mid-shaft of the right femur of each animal under aseptic conditions. Surgery was performed under general anaesthesia induced with 5% halothane (May and Baker UK), 25% oxygen and 75% nitrous oxide and maintained with 2.5–3% halothane, 25% oxygen and 75% nitrous oxide. An incision was made in the skin over the right femur and the mid-shaft of the bone exposed by means of sharp and blunt dissection. A defect was created in the bone using a 1mm diameter round stainless steel dental bur running at slow speed in a dental handpiece under saline irrigation. One implant was placed in the defect and the wound closed with sutures. An intra-peritoneal injection 0.05 ml oxytetracyline (Terramycin LA, Pfizer) was administered prior to recovery.

At 28 days animals were killed using a schedule one method. The right femurs were dissected free and placed in 3% gluteraldehyde in 0.1M-caccodylate buffer.

2.4. Scanning electron microscopy (SEM)

Specimens were dehydrated through ascending grades of alcohol and embedded in resin (LR White hard grade resin, London Resin Company, Reading, UK). Each resin block was trimmed with a fret saw and file to expose the implant and then polished with graded aluminium oxide lapping papers to produce a smooth, flat surface. Blocks were vacuum-coated with carbon and viewed using a Philips PSEM 501B scanning electron microscope in backscatter mode. All sections were examined and photomicrographs were later scanned to disk for use in image analysis.

Energy dispersive analysis by x-ray (EDS) examination of bone adjacent to the rods was carried out to search for metal ions within bone, the probe was applied at sites within bone in close proximity to implants and at sites further away. The probe was also applied to the surface and bulk of the implants.

Additional unused implants were vacuum-coated with gold and viewed to examine and compare the surface topographies of the implants.

2.5. Light microscopy

After examination with SEM, the specimen blocks were repolished with lapping paper to remove the carbon and then stained with Stevenel's blue and Van Gieson's picro fuchsin as described by Manitopoulous et al. [14], before producing ground sections 20–30μ thick. Sections were viewed through the slide using a Nikon Optiphot-2 microscope.

2.6. Profilometry

Short lengths of both coated and uncoated wires were examined to assess surface roughness using a Mitutoyo 301

Surftest (Mitutoyo Corporation, Kanagawa, Japan), eight readings were taken from one sample from each of the test implant types and an average value used to determine Sa values.

2.7. Image analysis

Analysis was undertaken using Image Pro-Plus (Media Cybernetics, USA). Osseointegration was calculated as the ratio of the length of the implant side in intimate contact with bone to the length of implant side and expressed as percentage. The cut ends of the rods were not included in the measurements. The mean value was calculated for each material and differences between the groups determined using the student t-test. Osseoconduction was calculated as the ratio of the length of rod side along which bone had appeared to grow, but was not necessarily contacting, to length of rod side; values for osseoconduction were also expressed as a percentage. Ground sections were viewed with the light microscope attached to a camera and computer so that images could be assessed using Image Pro-Plus, percentage osseoconduction and osseointegration were similarly calculated for ground sections.

3. Results

3.1. Scanning electron microscopy

In all cases, there was evidence of bone around the metallic implants and the rods appeared to have integrated with bone. Where rods were situated within the cortical region of the femur, bone had grown up to and was in close apposition to the metal. Where the rods had projected beyond the periosteal surface, there was a funnel-shaped defect between implant and bone (Fig. 1c). It was not possible using the scanning electron microscope to identify the nature of the material within this, but it but it was less dense than either the bone or the implant and may have represented soft tissue growing down the rod from the overlying soft tissue. Where rods projected into the marrow space, bone was seen alongside the rods, apparently extending from the cortical region, and the width of this bone reducing with increasing distance from the cortex. In some cases there was evidence of trabecular bone growth; this was seen mainly in the stainless steel group and to a lesser extent in the c.p.Ti group (Figs 1a and c).

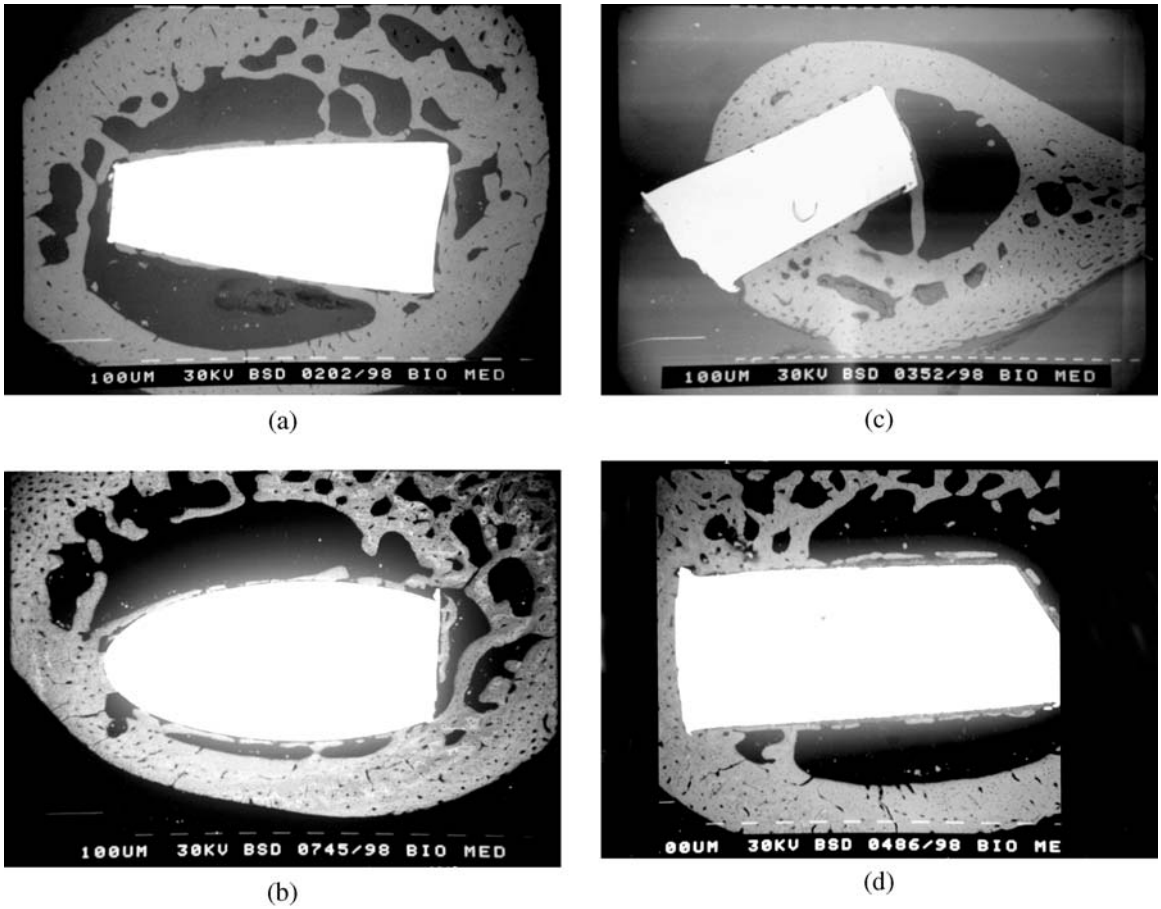

Fig. 1 Scanning electron micrograph of implants within bone; stainless steel (a), TAN coated stainless steel (b), commercially pure titanium (c) and TAN coated commercially pure titanium (d). Bars on image represent 100μm, i = implant.

It was noticeable that the bone growing around the rods was not always in direct, intimate close contact with the metal and appeared to have pulled away from the implants (Fig. 1b and d), this was assumed to represent shrinkage artefact produced during processing. The space between bone and metal was sometimes occupied by debris; this appeared to have originated from the lapping paper used for polishing since examination using EDS demonstrated the presence of aluminium. In other cases the space was occupied by amorphous material that could not be identified using EDS and was presumed to be soft tissue or resin.

Scanning electron microscope examination of the surfaces of coated and uncoated rods showed that coated rods had similar surface morphologies to their uncoated counterparts and that the stainless steel rods were apparently smoother than the c.p.Titanium rods. Small surface defects in the coating were occasionally seen.

3.2. EDS

When the EDS probe was applied to the bone close to SS rods, iron was detected in three cases, in two of these nickel was also seen and chromium in one of these two. In the remaining four cases there were no metal ions detected in the bone close to the rods and in none of the specimens at sites remote from the rods. Titanium and aluminium was detected in the bone close to the bone in four of the coated stainless steel samples. Iron was seen in three of the four and magnesium on one of the three. In the latter specimen aluminium and magnesium were also seen. Titanium was detected in bone close to the c.p.Titanium rods in four specimens, none was seen remotely from the rods and no other metallic ions were detected in the bone. Titanium was detected in bone close to rods in two of the specimens implanted with coated c.p.Titanium, in these cases and a further two specimens aluminium was also found in the bone close to the rods. None of these metals were detected at remote bone sites.

3.3. Light microscopy

Light microscopic examination revealed an essentially similar picture to that seen with the scanning electron microscope, in that all materials appeared to integrate well with no evidence of soft tissue encapsulation or chronic inflammation. The funnel shaped defect seen at the bone - soft tissue interface contained fibrous connective tissue. The apparent separation of bone from implant as seen on SEM examination was not so readily seen at this level, however; it was seen to a certain extent in two sections from the stainless steel group, one from the coated stainless steel and three from the c.p.titanium specimens. When present, the gap between the bone and rod appeared to be occupied a thin layer of cellular material that appeared most like marrow tissue, it was not possible to readily distinguish between two groups of tightly and loosely adherent bone as had been the case with the SEM images and for this reason the percentage bone around the rods was calculated as percentage osseoconduction, the results of which can be seen in table 1.

Table 1 Percentage osseointegration and osseoconduction for the four test materials +/− standard deviation. There is no significant difference in osseoconduction between the materials when assessed using either SEM or light microscopy. When percentage integration was measured on SEM images the amount of new bone in close contact with rods was significantly less for coated rods when compared with the plain rods, ($p < 0.01$ ss:TANss and $p < 0.01$ cpTi:TANcpTi).

	ss	TANss	cpTi	TANcpTi
%-conduction SEM	69%+/−11	63%+/−24	70%+/−15	64%+/−12
% integration SEM	50%+/−17	26%+/−12	53%+/−15	11%+/−7
% conduction ground section	70%+/−26	66%+/−17	66%+/−14	64%+/−20

3.4. Profilometry

The Ra values as measured using the profilometer were fairly consistent within any one test wire, but gave an occasional extreme value, the c.p. titanium gave the most consistent results with an average Ra value of $1.368 + / - 0.163$, coated titanium had an average Ra value of $2.507 + / - 0.913$, but when extreme readings were excluded this became $2.05 + / - 0.319$. The average Ra values for coated and uncoated stainless steel were $0.425 + / - 0.215$ and $0.92 + / - 0.173$ respectively.

3.5. Image analysis

Image analysis was carried out on digitised photomicrographs and ground sections viewed directly. Where a space existed between the implant and bone that was presumed to be shrinkage artefact the total length of bone presumed to be in contact was assessed and percentage osseoconduction calculated. When viewing ground sections, assessment of percentage osseointegration produced results very similar to the percentage osseoconduction as measured on the scanning electron microscope images (table 1).

There was no significant difference in osseoconduction that is, the amount of new bone growth along the rods, between the materials whether assessed from SEM images or from ground sections, but there was a significant reduction in the integration of coated rods for both materials with $p = 0.017$ for coated versus uncoated stainless steel and $p = 0.0085$ for coated versus uncoated c.p.Titanium.

4. Discussion

The scanning electron microscope in backscatter mode allows identification of materials of different electron densities, specimen preparation is straightforward and the technique provides an easy means for assessment of integration of implants within bone and furthermore, material can be reprocessed for light microscopy. The images produced are two dimensional and lack the error associated with depth of field problems seen in ground sections. However the technique does not allow examination of cellular detail.

The examination of a two dimensional image of a flat surface without the problems of depth of field of view as experienced with some of the ground sections may account for the apparent difference in the attachment of bone to metal rods when examined using the two microscopes.

SEM examination showed the stainless steel wire to be smoother than the titanium wire and this was confirmed by the surface roughness analysis. Surface roughness has been shown to be important for osseointegration [1, 3].

EDS enables identification of elements within specimens, but drawbacks to the method include variable depth of penetration of probe and lack of corresponding depth of view of the image on the screen. The finding of metal ions within the bone close to the rods may have been due to increasing curvature of the rod in the deeper parts of the specimen being detected by the probe and may not represent presence of metals within the bone although of course this too is possible and would suggest that metal had leached out of the rods or that corrosion had occurred and that fragments may have broken away from the main body of the specimen. There were no visible metal fragments seen in SEM or in ground sections, however accurate quantification of metal loss into surrounding tissue would require more sophisticated analytical techniques. At present it would appear that the coatings have not deteriorated over the time period although longer term studies would be required to assess the durability of the TAN coating in vivo, however, in view of the performance of this and similar coatings on high speed cutting tools it seems unlikely that these coatings would become detached from the underlying metal, though the adhesion to different metallic substrates may vary.

The apparent difference in integration between the coated implants and their uncoated counterparts is difficult to explain on the basis of the evidence gathered in this study. The surface roughness and contour did not differ markedly between the coated and uncoated partners, nor was there any difference in soft tissue response. The appearance seemed consistent with shrinkage due to processing but was notably more marked on the coated implants which suggest the surface chemistry is a significant factor and may influence cell adhesion and development of osseointegration.

The presence of a TAN coating had no significant effect on the amount of new bone growth around stainless steel and commercially pure titanium implants in the rat femur healing model of bone repair, however, the quality of osseointegration to the coating may be reduced.

The TAN coating may be easier to clean if cell adhesion is reduced, e.g. bacterial plaque on intra-oral abutments, or indeed, on fixtures that are exposed in the mouth.

The TAN coating may also be useful on temporary implanted materials such as the cover screws on dental implants where bone bonding is not required.

The TAN coating is biologically acceptable in the model used and therefore could be considered as a coating on to less acceptable materials which have good mechanical properties, but which may corrode in use or provoke a chronic inflammatory response.

5. Conclusions

The TAN coating is biocompatible in bone in the model used, with no significant effect on the amount of new bone growth around stainless steel and commercially pure titanium. The integration of bone with the TAN coating may be weaker than the bond with the uncoated metal.

TAN coating may be useful in an intra-oral environment or in percutaneous applications because the hardness of the surface is likely to make them less susceptible to damage. If there is less secure cell adhesion then these coatings may enable easier cleaning when exposed in the mouth however the nature of bonding with soft tissues requires investigation

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