Controlled Laser Texturing of Titanium Results in Reliable Osteointegration

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Author Contributions:

The authors Li, Blunn, Garrod and Liu contributed to the study design. Authors Li, Garrod, Mirhosseini and Liu prepared the laser-etched surfaces. Animal surgery, data acquisition, analysis and interpretation of data was performed by Blunn, Erskine and Coathup. The manuscript was drafted by Coathup and Blunn and all authors have read and approved the final submitted manuscript.

ABSTRACT

We have developed a laser-textured superhydrophilic Ti-6Al-4V surface with unique surface chemistry and topography that substantially promotes osteoblast adhesion in culture. Here we investigate the osteointegration of laser-textured implants in an ovine model. Our hypothesis was that laser-textured implants, without any surface coating (LT), would encourage comparable amounts of bone-implant contact and interfacial strength when compared with widely accepted hydroxyapatite (HA) coated implants. Additionally, we hypothesized that LT would significantly increase bony integration compared with machine-finished (MF) and grit-blasted (GB) implants. Forty-eight tapered transcortical pins were implanted into six sheep. Four experimental groups (LT, HA, MF and GB) were investigated($n = 12$) and implants remained *in vivo* for 6 weeks. Bone apposition rates, interfacial shear strength and bone-implant contact (BIC) were quantified. The interfacial strength of LT and HA implants were found to be significantly greater than GB ($p=0.032$ and $p=0.004$) and MF ($p=0.004$ and $p=0.004$ respectively), but no significant difference between LT and HA implants was observed. Significantly increased BIC was measured adjacent to HA implants when compared with both LT and GB implant surfaces ($p=0.022$ and $p=0.006$ respectively). No significant difference was found when LT and GB implants were

compared. However, all surface finishes encouraged significantly increased BIC when compared with the MF surface.

Clinical Significance: Maximising implant fixation to host bone is vital for its long-term success. The production of an LT surface is a simple and cheap manufacturing process and this study demonstrated that laser-textured implants are a very promising technical development that warrants further research.

Keywords: Laser-textured; osteointegration, hydroxyapatite; animal model; bone regeneration.

Introduction

Over 160,000 hip and knee replacements were undertaken in England and Wales in 2011, accounting for a significant proportion of NHS resources (1). Data from an American national database showed that 46% of total hip arthroplasties (THAs) are now being performed in patients under 65 years of age, and this is predicted to increase to 55% by 2015 (2). With this change in patient demographics there is a requirement for enhanced implant longevity, which requires optimisation of bone fixation.

Cementless fixation uses surface coatings or textured ongrowth surfaces to invoke new bone growth at the implant interface leading to biological fixation through osteointegration. This biological process permits regeneration and remodelling over time and also allows for the adaptive capability of bone to respond to any changes in the patients' biomechanical environments (3). Implant osteointegration is key to increasing longevity of an uncemented implant in orthopaedic surgery and the choice of ingrowth surface used is crucial as it affects

long-term survival (4). It has been postulated that a porous structure, has a role in stimulating bone formation and preventing bone resorption (5). Optimum bone growth is associated with a pore size ranging from 200 – 400 μm (and a minimal pore size of 50 to 150 μm) (6). A successful surface coating in terms of achieving osteointegration has involved the addition or alteration in quantities of inorganic phases such as calcium and phosphate (7). Calcium phosphate coatings replicating the mineral phase of bone are bioactive and able to bond directly with bones, thereby increasing implant interfacial strength (8). A study by Coathup et al. (9) investigated uncemented femoral components retrieved from 58 patients at post-mortem and compared bone growth to a hydroxyapatite (HA) porous coating with an uncoated plasma-sprayed porous coating and a grit blasted surface finish. This study reported increased and more evenly distributed bone in direct contact with the HA coated porous surface when compared with the porous only and grit blasted surfaces. Animal studies have further supported these findings where HA coated implants increased direct bone-implant contact (BIC) and increased torque by four times when compared with non-coated grit blasted surfaces (10, 11). HA coated surfaces have also been shown to be more resistant to migration of wear debris along the interface (12). Despite showing excellent long-term clinical results, there are concerns regarding HA's resorption over time and concomitant exposure of the underlying titanium surface, which is reported to decrease bone-implant contact in the years immediately post-surgery (13, 14). Delamination of HA has also been reported for thick coatings where there is a separation between the alloy surface and the plasma sprayed HA surface and this is believed to be associated with the difference in stiffness between these materials. HA particles have been shown to elicit an inflammatory response and are reported to contribute to third-body wear, osteolysis and eventually aseptic loosening (15).

Various techniques such as grit blast and acid etching have been used to roughen the implant surfaces in order to improve the clinical performance and to encourage a stable mechanical bone-implant interface. However, sand blasting is reported to leave contaminations on the implant surface, which is not desirable (16). On the other hand, both grit blast and acid etch produce surfaces with poorly-defined topography and various chemical compositions and the optimal surface properties which result in rapid osteoblast attachment, proliferation and differentiation are not yet established.

Laser texturing has been developed over the past decade to roughen the implant surface by producing hemispherical pores and this process has been used successfully to augment dental implant fixation (17). The process evaporates the original surface layer and re-shapes a desirable micro morphological topology with enhanced surface roughness and chemical compositions to promote osteointegration. The resulting surface roughness is similar to that produced by acid-texturing and replicates the texture of the bone matrix, and has been shown to enhance osteogenic cells to form new bone (6, 18). Single wavelength pulses are fired at a particular spot on the surface, heating the material to a high enough temperature to cause a phase transition. As the material cools again the titanium alloy re-solidifies leaving behind both micro- and nano-indentations. This process modifies the surface topography and wettability without significantly altering its surface chemistry and laser texturing is advantageous to alternative processes as it is site specific, causes less disruption to the titanium's purity and is both clean and repeatable (19).

Various surface textures have been created on the surface of biomaterials including uniform and non-uniform patterns. An example is using a femtosecond and a picosecond laser to create uniform and repeatable patterns on a desired portion of the surface on Ti-6Al-4V. They found that different surface patterns could enhance or inhibit human osteosarcoma cell

attachment (20). Mirhosseini et al. reported that laser surface patterning of small holes on Ti–6Al–4V samples and the synthesis of Ca_2SiO_4 coating on Ti–6Al–4V samples by laser irradiation under submerged conditions would both encourage the 2T3 osteoblast cell growth (21, 22). Mukherjee et al studied the effect of laser created grooves with different widths and depths on MG63 cells. They have used a continuous wave fiber laser and showed that surface features dimensionally closer to the cell dimensions were able to positively affect the viability and spreading of MG63 cells (23). Laser surface irradiation of Ti-6Al-4V samples by using a High Power Diode Laser (HPDL) was reported by Hao et al to increase the wettability and osteoblast response (24). Hallgren et al. produced 10 µm hemispherical pits with a pitch of 30 µm on titanium dental implants by using a Nd:YAG laser and reported significantly more bone-to-implant contact in rabbit femur and tibia in a twelve week study (25). The above investigations only focused on the laser generation of surface structures and their in vitro effects on biocompatibility, fewer studies have investigated these surfaces in vivo. There are a number of different surfaces available for osteointegration. The advantages of using a laser textured surface are that it produces a surface of intermediate roughness, there is minimal contamination, the porosity that is created is part of the implant and therefore delamination does not occur.

This study aimed to investigate the osteointegration of our novel laser-textured implant in an in-vivo ovine trans-cortical pin model. The laser-textured super-oxidized surface investigated had a novel uniform spaced parallel ridge pattern. Our hypothesis was that implants modified using our laser-texturing technique (LT) would encourage comparable amounts of bone-implant contact and interfacial strength when compared with hydroxyapatite (HA) coated implants. We further hypothesized that an increased amount of bony integration would be

measured adjacent to LT implants when compared with machine-finished (MF) and grit-

blasted (GB) implants.

Materials and Methods

In order to minimise the number of animals required in this study, a power analysis based on

our previous work where a significance of p=0.05 showed that the minimum number of

animals to achieve a significant difference with a power of 0.8 was 6 in order to observe a

20% difference between control and experimental groups, which is required for

translational/clinical impact. A total of 48 tapered pins measuring 5 – 4 mm in diameter and

20 mm in length were manufactured from titanium alloy (Ti-6Al-4V). Twelve implants were

assigned to each of the four experimental groups which were; 1) Grit blasted (GB), 2)

Machine-finish (MF), 3) Plasma sprayed HA coated and 4) Laser-textured (LT). All implant

surfaces (apart from those in the LT group) were prepared by Plasma Biotal, (Tideswell,

Derbyshire, UK). Surface roughness was quantified in all groups except the LT group, using

three-dimensional non-contact analyses and Optical Microscopy (Contour-GT, Bruker, MA,

USA). Four equi-distant measurements were taken along the length of each of the surfaces

and results compared. The MF surface investigated had a mean surface roughness (R_a) of 0.1

µm, the GB surface 6 µm and the HA coated surface a roughness of 2.0 µm. Implant surfaces

in the grit blasted and plasma sprayed HA coated group were blasted with aluminium oxide

beads of 5 microns in size. Implants in the plasma sprayed HA group, were additionally

coated with a highly crystalline (> 85%), thin (<70 µm) hydroxyapatite coating. Implants

were gamma sterilized prior to insertion during surgery, however prior to sterilization and HA

coating, implants in all groups were ultrasonically cleaned in Decon 90™ (Decon

Laboratories Limited, Hove, UK) for 20 minutes, followed by washing in distilled water,

isopropanol and trichloroethylene, each for **20 minutes**. Implants were inserted into 6

skeletally mature, commercially crossbred, female sheep weighing 70 – 90 kg. A 100 mm

incision was made over the medial aspect of the tibia and the bone exposed using blunt

dissection. The periosteum was removed and bi-cortical defects created using a drill and tap

prior to implantation of the pin. Implants were separated by at least 1.5 cm of bone and four

pins were inserted into both the left and right tibiae (a total of 8 implants per animal) (Figure

1). Implant positions were rotated such that no implant combination was placed in the same

location in each sheep more than once. All procedures were carried out following Ethics

approval granted by the Royal Veterinary College and in compliance with the United

Kingdom Home Office regulations [Animal Scientific Procedures Act (1986)]. Following

surgery, animals were allowed immediate and full mobilization as tolerated. Antibiotic and

analgesic prophylaxis were administered daily with subcutaneous injections of Baytril

(Enrofloxacin 5 mg/kg; Bayer AG Leverkusen) and Finadyne (Flunixin Meglumine 2 mg/45

kg; Schering-Plough) for 3 days post-surgery. Animals were kept indoors and in individual

pens for 1 week post operatively before being group housed. The general health of all

animals was observed and recorded daily, and transfer of animals from one housing regime to

another was dependent upon recovery from the surgery, and was therefore made on an

individual basis. Two fluorochrome bone markers Oxytetracyline (30 mg/kg) and Calcein

Green (30 mg/kg) were administered at weeks 3 and 5 post surgery. Oxytetracylcine and

Calcein Green localize at sites of mineralization and, when viewed under ultra-violet light,

fluoresce orange and green, respectively. The mean measurable distance between cement

lines provided an assessment of bone apposition rates (μm/day) adjacent to pins in each of the

groups. Implants remained *in vivo* 6 weeks and animals were euthanized following

administration of sodium pentobarbital (Euthatal, 150mg/kg; JM Loveridge Plc, Hampshire,

UK). This time-point was chosen as it is considered a clinically relevant time-point where

bone formation has been shown to occur.

Within 8 hours of retrieval, the interfacial shear strength of 6 implants in each experimental group were assessed using a Zwick ProLine 500 (Zwick/Roell, LTominster, Herefordshire, UK) pull-out testing machine. The remaining 6 implants in each group, were placed in 4% paraformaldehyde solution before being processed for undecalcified histology. Following dehydration in serial dilutions of alcohol, specimens were defatted and embedded in hard grade acrylic resin (LR White, London Resin Company, Reading, UK). Thin sections (~ 70 µm) were prepared by making longitudinal cuts through the centre of each pin using a grinding and polishing technique (EXAKT, Norderstedt, Germany). Samples were stained with Toluidine Blue and Paragon, which stained the soft tissue and bone respectively. Bone contact was assessed along the entire bone-implant interface of each of the pins using image analysis software (Axiovision Release 4.6, Carl Zeiss, Jena, Germany). The ridged surface of the LT implant demonstrated bone growth between the ridges and onto the surface of those ridges. This allowed us to measure the amount of bone between the ridges. Eight random regions of interest (ROI) were image captured along the implant interface and assessments made using image analysis software to quantify the proportion of mineralised bone that had integrated within the structure.

Laser Surface Texturing

A diode pumped Nd:YVO_4 laser with a wavelength of 532 nm, a pulse duration of 8 ns and a repetition rate of 30 kHz was used to texture the implants in a N_2 gas environment. The laser beam with a near Gaussian intensity distribution ($M^2 \sim 1.5$) was focused onto the surface with a spot diameter of ~ 55 µm.

The laser beam was raster scanned at a speed of 20 mm/s over the surface of the implants in parallel scanning lines with a hatch distance of 20 µm, using a computer controlled galvo-

scanning system equipped with a flat field lens. This produced a ridge height and width of 56

and 20 **µm** respectively with a spacing of 40 µm. This also has a structure on the ridges,

which has a roughness in the micron range (Figure 2; Table 1).

Interfacial Shear Test

Interfacial shear strength measurements were carried out in accordance with ISO-13779-4. A

M3 thread located within the implant was attached to a Zwick ProLine 500 (Zwick/Roell,

Leominster, Herefordshire, UK) testing machine, and a pre-load of 1 N/mm^2 and a test speed

of 5 mm/min applied. Force-deformation curves were recorded and the maximum force (F-

max) was used to represent the maximum strength of the bone-implant interface.

Backscattered Scanning Electron Microscopy

Thin sections were sputter-coated with a layer of gold palladium and viewed using

Backscattered electron microscopy (JOEL JSM-5500LV; Jeol, Welwyn Garden City, UK).

Statistical Analysis

Analysis of the data was performed using SPSS software (v10.1; SPSS, Chicago, Illinios,

USA). A Kolmogorov-Smirnov test showed the data obtained were non parametric and the

Mann-Whitney U test was used for statistical comparison between experimental groups. A

Pearson's *r* correlation coefficient was used to assess the relationship between the mean bone-

implant contact (BIC) and the **mean** interfacial shear strength for each group. Individual

implants were unable to be compared as data for both BIC and shear strength was obtained

from different implants as measurement techniques were destructive. *P* values < 0.05 were

considered significant.

RESULTS

All animals remained healthy throughout the duration of the study.

Bone Apposition Rates

Results (± standard deviation (SD)) showed no significant difference when each of the experimental groups were compared. The highest bone apposition rate was measured in the GB group (mean, 1.92 ± 0.20 μm/day) and the lowest was observed adjacent to LT implants (mean, 1.67 ± 0.22 μm/day) (Figure 3). A mean of 1.79 ± 0.25 μm/day was measured in the HA group and 1.93 ± 0.19 μm/day in the MF implant group.

Bone-Implant Contact

Bone-implant contact adjacent to HA coated pins was significantly increased (mean, 64.85 ± 10.18%) when compared with each of the other surface finishes (LT mean, 36.81 ± 9.61%, p = 0.022; GB mean, 36.79 ± 11.15%, p = 0.006; and MF mean, 15.07 ± 5.61%, p = 0.006). Significantly increased bone-implant contact was measured in the LT and GB groups when compared with the MF surface finish (p = 0.004 and 0.006 respectively). No significant difference was found when LT and GB implants were compared (Figure 4).

Bony Ongrowth into the LT Surface

Due to the nature of the surface, bone ongrowth on LT implants showed that a mean of 48.78 ± 6.15% of the surface had grown on and within the ridges. In the LT group, BSEM showed direct bone growth within the ridges (Figure 5). Osteoconduction along the HA surface was observed where more bone appeared on the HA surface where the pin transected within the medullary canal. This was also seen to a lesser degree in the LT group. Backscattered

scanning electron microscopy showed that in the MF group, bone growth was seen immediately adjacent to the implants. However in most places, bone did not make direct contact with the surface.

Interfacial Shear Strength

The maximum force (F-max) sustained before the pin was detached from the surrounding bone was recorded. The highest measure of interfacial shear strength was seen in the HA coated group (mean, 2611.16 ± 306.54 N) (Figure 6). A significantly higher interfacial strength was measured in the HA group when compared with all groups except the LT group (mean, 2265.26 ± 751.23 N; GB mean, 1199.89 ± 444.77 N, $p = 0.004$; MF mean, 183.05 ± 151.05 N, $p = 0.004$). A significantly higher interfacial strength was measured in the LT samples when compared with both the GB ($p = 0.032$) and MF ($p = 0,004$) groups. Additionally, significantly increased interfacial shear strength was measured adjacent to GB implants when compared with MF implants ($p = 0.004$). It was evident that failure during pullout testing in the MF and GB groups occurred along the bone-implant interface. Whereas for the LT group, failure occurred within the bone with bone tissue identified within the LT ridges. For the HA implants, failure occurred both within the bone and along the HA-metal interface exposing parts of the underlying titanium alloy (Figure 7)

Correlation analysis showed a strong relationship between BIC and interfacial shear strength ($r = 0.88$) (Figure 8a). Results showed that LT implants fell below the trend and when removed from the analysis. A stronger correlation of $r = 0.99$ was found (Figure 8b). This suggested the LT grooved surface increased the force required to remove the implant from bone despite the lower BIC value.

Histology

Qualitative analysis using optical microscopy showed that all surface finishes investigated, including MF, demonstrated direct bone contact to the implant surface. However, implants coated with HA showed more continuous bone-implant contact. Although direct bone-implant contact was seen in the GB and LT groups, there were many areas where bone was interrupted by a soft tissue layer interfacing the implant surface (Figure 9). Although osteogenesis had occurred in the MF group, the bone was separated from the underlying titanium alloy surface by a thin layer of fibrous tissue.

DISCUSSION

The bone-implant interface was investigated both qualitatively and quantitatively using biomechanical testing, histology and backscattered SEM. Results showed a significant increase in the levels of BIC with HA, LT and GB compared to MF, and when HA was compared to both LT and GB. However, our study did not find a significant difference when LT and GB surfaces were compared. These findings were also reflected in the biomechanical test results, with the exception that the interfacial strength at the LT interface significantly increased when compared with GB surfaces. Our results therefore do not support the first part of our hypothesis, that a laser-textured technique would encourage comparable amounts of bone-implant contact when compared with hydroxyapatite (HA) coated implants. However, we can accept part of our second hypothesis as increased bony integration was measured adjacent to LT implants when compared with the machine-finished surface.

Results showed that BIC measured in the HA group (65%) was almost twice that measured in both the LT (37%) and GB (37%) groups. A study by Abeli et al. (26) measured a similar amount of BIC and reported that significantly increased osteointegration occurred adjacent to

a HA coated implant (68%) when compared with a non-coated vacuum plasma sprayed titanium implant surface (46%) in an ovine model at 4 weeks post-implantation. Similarly, an intramedullary rabbit model reported significantly increased BIC (64%) to a HA coated implant 6 weeks post-implantation when compared with a non-coated chemically-textured titanium surface (39%) (27). Our pull-out test data is also comparable with studies reported in the literature (28) and Aebli et al. (26) showed HA to have a significantly superior interfacial strength when compared to a GB control. Results from our study also showed no evidence of HA delamination, resorption, or an inflammatory response along the interface whilst the implants were *in situ* confirmed the effectiveness of HA in bony regeneration and osteointegration when compared with non-coated titanium surfaces. We did, however, show that delamination of the HA coating occurred during pull-out tests. This exposed the underlying alloy surface, which was also exposed when MF and GB implants were pulled from the bone. However, failure within the bone occurred for the LT implants.

Previous studies have concluded that rougher surfaces increase BIC along the interface (6, 29). However, our findings do not support this conclusion as our results showed that mean BIC values were similar in the GB and LT groups. A study by Stangl et al. (30) failed to observe any advantage of using a laser-textured surface with a pore size of 50 μm (14%) over a grit-blasted surface (23%) in a rabbit intramedullary model 6 weeks post implantation. A study by Prodanov et al. (31) also showed no significant difference when bone-to-implant contact to a laser-treated surface (68%, ±17) was compared with a grit blasted/acid etched surface (49%, ±21) when inserted in a rabbit model at 8 weeks post-implantation. There are many studies that support increased osteointegration when compared to a MF control. Branemark et al. (32) reported that a LT implant significantly augmented BIC (26.5%) when compared to the MF control (18%). Paz et al. (19) similarly found BIC at the LT surface to be

significantly increased (84%) when compared with a MF control (35.9%) following a 3 month implantation period. Although studies by Aparicio et al. (33) and Guo et al. (34) reported a 40% BIC rate to a MF surface, our results showed lower BIC and were similar to other studies where bone contact measured in the range of 10 - 20% (32, 35, 36). A study by Faeda et al. (37), investigated the interfacial shear strength of LT modified surfaces in a rabbit transcortical pin model. Results showed that 4 weeks post-implantation the laser modified implant was associated with a significantly higher removal torque of 3300 N/mm^2 compared to the MF control (2328 N/mm^2). Studies by Branemark et al. (32) and Palmquist et al. (38) also reported that a LT surface augmented osteointegration and a significantly higher removal torque was measured when compared with a MF control at 8 weeks and 6 months respectively post-implantation.

The inconsistency seen between pull-out test results and BIC has also been reported in previous studies. In our study, a Pearson's correlation coefficient of 0.88 was measured when comparing the means of each group. This indicates a strong positive relationship between these parameters and is comparable with previous studies (39, 40). However in our study, the relationship of BIC and interfacial strength at the LT interface did not follow this trend with stronger interfaces for a given amount of bone-implant contact being seen with the LT implants compared with the HA, MF and GB groups. In the HA, GB and MF groups, there was a very high correlation between BIC and interfacial shear strength. This showed that for every 1% of BIC there was a concomitant increase of approximately 40 Nmm^2. However, in the LT implant group, for every 1% increase in BIC there was an increase of approximately 60 Nmm^2. The higher than anticipated interfacial strength given the level of measurable BIC achieved could be due to a number of possibilities. The higher interfacial strength at the LT surface suggests the quality of bone anchorage may have been greater than seen with its

comparator GB. Bone growth onto the LT surface was measured in our study and results showed direct contact with 48.8% of the ingrowth surface occupied by bone. The larger amount of bone ingrowth on an LT surface may be associated with a more biological response due to bone formation being enhanced between the ridges. The structural nature of the LT surface allowed bone to interdigitate with the implant leading to greater resistance to shear. It is known that rougher surfaces promote osteogenic cells to form new bone around the implant (41). Cooper et al. (6) described the ability of osteoblastic cells to sense the grooved LT surface with the aid of integrins, which then bind to proteins adsorbed to the titanium surface. It has also been reported that laser-engineered titanium surfaces significantly increase the viability, adhesion and proliferation of osteoblastic cells (18, 42). An *in vitro* study investigated LT discs and measured a decrease in the attachment of early inflammatory cells (38). The authors postulated that this decrease was related to the LT surface replicating similar nanometre features resulting in increased extracellular matrix protein attachment.

Maximising the potential for initial stability and good fixation of an implant to the host bone is vital for its long-term success. In this study the degree and strength of osteointegration associated with different surface implants was assessed using an ovine model. Our study investigated osteointegration in healthy cortical bone using tapered pins. The rationale for this was that this is a comparative study of different surface textures and relative differences can be measured using this approach where variability associated with insertion and cancellous bone structure can be limited. Cortical bone models have been used previously to study the interaction of implants with different materials (43, 44). Results showed that HA encouraged significantly increased bone-implant contact when compared with LT, GB and MF surface finishes. In terms of their function, LT samples showed no significant difference in their bone attachment from HA implants and were more strongly attached than either GB

or MF implants. Although BIC to the HA surface was significantly higher, the increase in surface roughness of the LT implants allowed better bone growth which resisted the forces applied during the pull out tests giving an interfacial strength similar to the smoother HA coated surface. The LT surface did not appear to be more bioactive as a result of changes to surface chemistry and the reasons for this remain unclear. The production of an LT surface is a simple and cheap manufacturing process and this study demonstrated that laser-textured implants are a very promising technical development that warrants further research in larger studies.

Acknowledgements

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Figure Legends

Figure 1.

A photograph showing the positioning of the four implantation sites along the diaphysis of the tibia.

Figure 2.

Photomicrographs taken using scanning electron microscopy showing [A] the laser textured surface, [B] an untreated Ti6Al4V surface, [C] a plasma sprayed hydroxyapatite coated surface and [D] the grit blasted surface.

Figure 3.

A box-plot illustrating bone apposition rates (μm/day) between in each of the experimental groups investigated.

Figure 4.

A box-plot showing bone-implant contact in each of the implant treatment groups.

Figure 5.

An electronmicrograph showing bone growth to a MF pin [A] and LT pin surface [B].

Figure 6.

A box-plot of ultimate interfacial shear strength in each of the experimental groups.

Figure 7.

A photograph showing HA coating delamination following mechanical testing.

Figures 8a and b.

Scatterplots showing the relationship between mean BIC and Interfacial Shear Strength.

Figure 9.

Cross-sectional micrographs of samples along the implant interface, (a) HA, (b) LT, (c) GB and (d) MF. Blue arrow indicates new bone growth, yellow arrow shows fibrous tissue in contact with the implant interface.