Biomechanical Evaluation of the Osseointegration of Biologically Coated Open-Cell Titanium Implants

Teodolito Guillén Giron, Arne Ohrndorf [*], Hans-Jürgen Christ

Institut für Werkstofftechnik, Universität Siegen, 57076 Siegen, Germany
* Corresponding author: arne.ohrndorf@uni-siegen.de

Abstract
The aim of this paper is to compare the osseointegration of open-cell titanium implants with or without the application of a biological $CaTiO_3$ reaction layer. Cellular implants on the basis of Ti6Al7Nb were precision cast and the brittle α-case was removed by means of a pickling process. Coated and uncoated specimens were then implanted into the distal femural metaphysis of sheep. After six months the sheep were euthanized in order to extract samples from the femural metaphysis. Half of the samples were used for histomorphological investigations and the rest of the samples were prepared for biomechanical testing. Prior to push-out testing the samples were aligned in the loading direction with the help of X-ray analysis. Compared to control samples the level of push-out loads increased by a factor of about 3-4 during the period of exposure to the living organism but no significant difference was measured between coated and uncoated specimens. SEM investigations revealed high amounts of cancellous bone formation inside the implants particularly in the main trabecular direction of the surrounding bone in both cases and mineralized bone adhesion at the pickled surfaces of the implants proving the excellent biocompatibility of both coated and uncoated implants.

Keywords titanium, implant, osseointegration, coating, push-out test

1. Introduction

For the coming decades a rising demand for orthopaedic surgery can be predicted due to the fact that in an aging population with increasing life expectancy the number of degenerative bone diseases treatments will get significantly higher. Typical bone diseases in the context of higher age comprise for example osteoporosis or bone cancer for which adequate medical treatments need to be developed. Apart from the degenerative diseases mentioned above trauma surgeons are looking for materials that can be used to substitute human cancellous bone that is damaged caused by accidents.
Nowadays, in such cases autologous bone material is extracted from the iliac crest [1] or from the proximal fibula rather than using allogenic material and subsequently implanted to substitute the damaged bone. The risks connected with the necessary additional surgical intervention [2, 3] are usually smaller compared to the potential problems of transmission of infectious allogenic material [4]. Artificial ceramic bone substitute materials on the basis of CaP or HA feature excellent biocompatibility but suffer from insufficient post-surgical mechanical properties [5]. Metallic bone substitute can help to overcome the problems of inadequate mechanical properties but require special surface treatments to achieve satisfactory biocompatibility. Titanium and its alloys are excellent candidates for implant applications because of their high corrosion resistance and good biocompatibility. A common material for orthodaedic implants is the two phase titanium alloy Ti-6Al-4V which offers superior mechanical properties than pure titanium. However, Ti-6Al-4V has become suspected to release vanadium under the physiologic conditions of the body leading to long-term toxicity [6]. By substituting vanadium with niobium in the alloy the problem of toxicity can be solved without significant losses in mechanical strength. Although titanium alloys present a remarkable intrinsic biocompatibility osseointegration of a titanium implant can be further improved by applying biological reaction layers on the substrate such hydroxyapatite (HA) [7] or calcium phosphate (CaP) [8].

Orthodaedic implants or bone substitutes made of metallic materials exhibit biomechanical incompatibility in terms of much higher stiffness compared to the bone material. Loads are hence mainly transferred by the implant and the bone around the implant degenerates. In the literature, this process is often called “stress shielding effect” [9] and can be prevented by adjusting the porosity of the metallic material and thus reducing the effective modulus of elasticity.

2. Experimental

2.1. Manufacturing of biologically coated open-cell titanium implants

2.1.1 Casting process

Open-cell titanium (Ti-6Al-7Nb) implants as shown in Figure 1 were manufactured using precision casting [10] at the Gießerei Institut in the RWTH Aachen University. The samples were fabricated by reproducing polyurethane templates which possess the desired final geometry of the samples. The polymer templates were filled with a mould casting slurry (invest Ti-T) based on MgO + Al_2O_3 + CaO which was then baked to harden the casting material. During the baking process, the polymer template was burning out and the subsequently free space left by the template removal was filled with the Ti-6Al-7Nb melt alloy with a centrifugal casting machine (TiCast Super R, SelecCast) which is depicted in Figure 2. The casting machine has a small electricarc furnace that produces a drop of molten metal lying on a skull of solid material, saving the melt from contaminations of the graphite crucible. While melting the ingot material, the plate on which the mould was fixed was accelerated to a final velocity of 3000 rpm to assure the complete filling of the mould. Casting of the Ti-6Al-7Nb parts was carried under an inert argon atmosphere at approx. 250 torr.

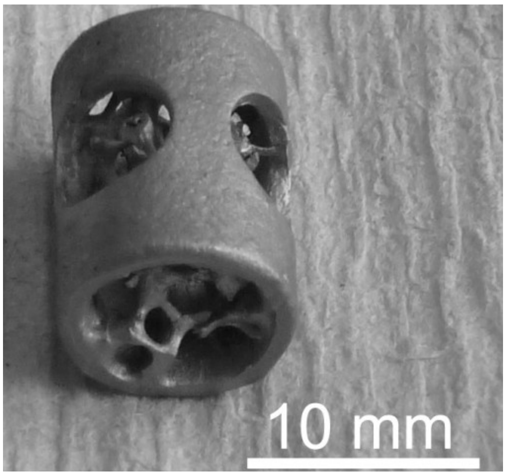

Figure 1: Open-cell titanium (Ti-6Al-7Nb) implants

The implants shown in Figure 1 were initially cast to a cylindrical shape of 10.5±1 mm and a length of 12.5±1 mm and consisted of a porous core structure with cell spacing of 1674±704 µm and strut thickness of 595±205 µm. In order to resist the implantation loads the implants possessed a dense outer ring interrupted by four holes to allow bone ingrowth into the open-cell core. Prior to implantation into the distal femoral metaphysic of sheep the cast implants were machined to an outer diameter of exactly 9.7mm and perfect cylindrical shape to ensure good contact to the surrounding bone.

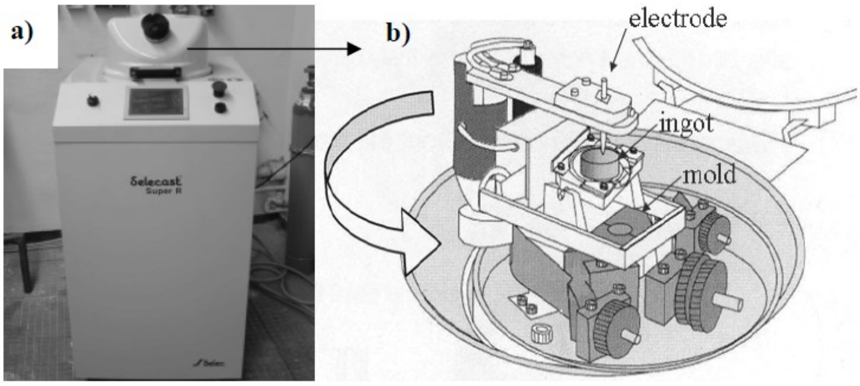

Figure 2: a) Centrifugal casting machine, b) schematic of the casting chamber

2.1.2 Heat treatment and surface modifications

In earlier work [11] the unfavorable microstructure resulting from the casting of sophisticated cellular structures made of Ti-6Al-7Nb was presented and discussed with respect to the bad mechanical behavior of cast specimens. In order to improve the mechanical properties of the open-cell implants a more homogeneous microstructure was established by aging at 600°C for four hours in an inert argon gas atmosphere. The most detrimental influence was attributed to the formation of a thick α-case layer due to reactions between the metal melt and the aluminium from the investment mold material during casting [11]. For all specimens implanted into animals hence a pickling process using an acid mixture of 70% HNO_3 + 10% HF in destilled water was developed which completely removed the brittle α-case layer after an exposure time of 60-70 minutes. Figure 3 shows the development of surface attack as a function of pickling time. At the end of the pickling process a "worm mark" structure on the surface reduced the roughness from R_{max}=45µm after casting to a value of R_{max}=9µm.

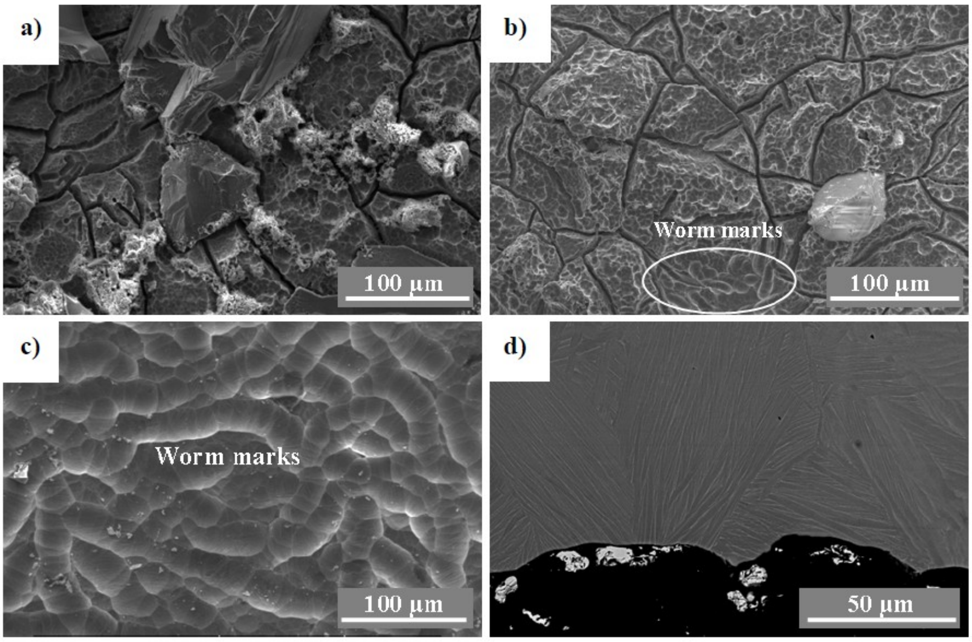

Figure 3: Pitting corrosion after: a) 20 min, b) 40 min, c) 60-70 min of pickling, and d) cross sectional SEM micrograph after 60-70 min of pickling

2.1.3 Coating with a biological $CaTiO_3$ reaction layer

After pickling, the samples were coated with calcium titanate reaction layer at the Bundesanstalt für Materialforschung und -prüfung (BAM) Berlin. Coatings were used to observe their effect in the osseointegration process. For this purpose, grid-shaped and plate samples were coated in different salt baths which are described in Table 1. The surface and thickness of the $CaTiO_3$ coatings SBI, SBII, SBIII and SBIV were characterized using SEM.

Table 1: Chemical composition of the different salt baths studied in this work

Code	Composition [Mol-%]			Temperature [°C]
	$Ca(NO_3)_2$	$NaNO_3$	KNO_3	
SBI	46	27	27	350
SBII	50	50	-	410
SBIII	60	40	-	450
SBIV	75	25	-	510

The performance of the coatings was evaluated using cyclic bending and fretting testing in Ringer's solution as well as SEM surface analysis and composition SBI was finally selected for the coating of half of the implant specimens.

2.2. Implantation of open-cell titanium implants in sheep

To analyse the biological reactions and biomechanical behaviour of the porous implants in the cancellous bone, they were implanted in sheep at the Justus-Liebig-Universität Gießen, Germany.
Sheep were used to study the osseointegration process and the mechanical behavior of the Ti-6Al-7Nb implants in the cancellous bone at the femoral metaphysis (Figure 4a) of in total 24 animals. Half of the implants were biologically coated with $CaTiO_3$ after the pickling process and the other 12 implants were only pickeled in order to compare the osseointegration of the two groups.
After 6 months, distal femoral metaphysis of 12 sheep (with ages between 49-95 months) with their respective implants (Figure 4b) were extracted and used for the in-vitro postoperative biomechanical test. The rest of the specimens were used for histomorphometric analysis at the Labor für Experimentelle Unfallchirurgie, JLU Gießen.

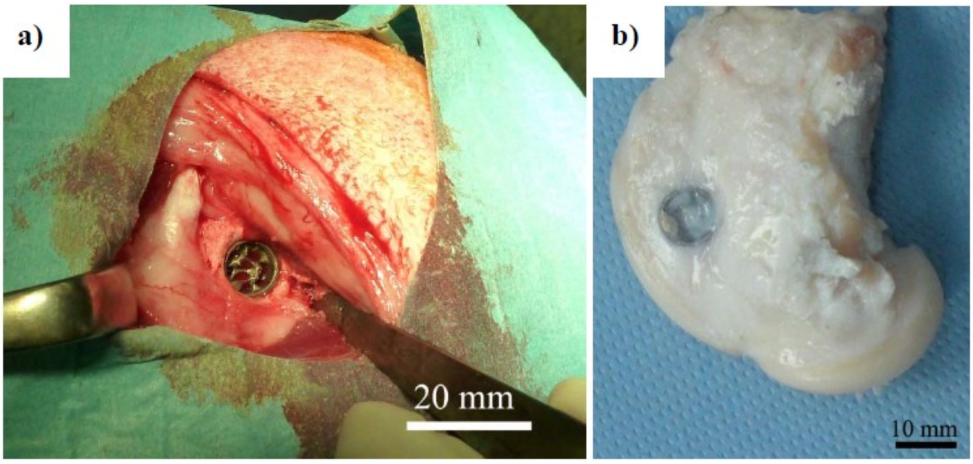

Figure 4: a) Implantation in an ovine distal femoral metaphysis, b) extracted bone with implant after 6 months

2.3. Biomechanical testing and histomorphological investigations

2.3.1 Biomechanical push-out testing

To analyse the mechanical characteristics of the bone-implant interface of sheep a push-out test was used. The samples used for the push-out test were carefully extracted from the femur of the sheep and laterally cut with a diamond saw. In order to use the pushout system showed in Figure 5 the cut side of the bones was carefully ground with an abrasive SiC paper of grit size 220 until the lower side of the implant was visible. To prevent misalignment of the implant during push-out testing X-ray analysis (Comet MRX 320/26 equipment at 50kV) of the implant specimens in two directions were carried out and the two inclination angles were determined prior to mechanical testing. The alignment screws visible in Figure 5 were then used to adjust the measured inclination angles and to align the specimen perfectly.

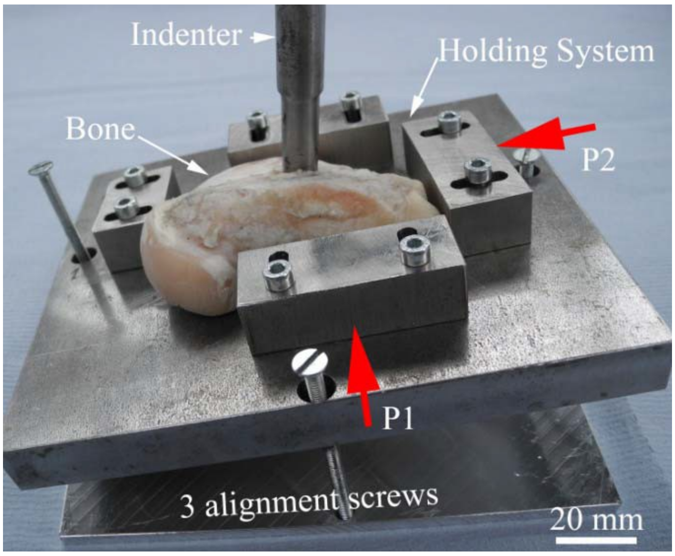

Figure 5: Push-out testing system used to analyse the ovine bone-implant interface

Push-out testing was performed on an MTS 810 servohydraulic system which was equipped with an indenter of 9.3 mm in diameter. The push-out force of the bone-implant interface was measured with a 50 kN load cell, at a displacement rate of 1 mm/min until the implant was completely removed from the bone. Force and absorbed energy of the bone-implant interface were registered and compared with the push-out test of the reference samples (specimens with 0 days of osseointegration).

2.3.2 Histomorphological investigations

Microstructure of the metallic and bone samples were analysed by using optical and scanning electron microscopy (SEM). SEM analysis was carried out with a Philips XL 30 equipment. Microstructure, failure analyses and changes in the surface topography were observed with secondary electron (SE) and back-scatter electron (BSE) detection in combination with energy-dispersive X-ray spectroscopy (EDS) and automated electron back-scattered diffraction (EBSD). A confocal laser microscope model Olympus LEXT OLS 4000 was utilized to measure the surface roughness of samples.
Prior to SEM analysis, bone samples were ultrasonically cleaned in ethanol at 50°C for 2 hr. Bone marrow, fat and oil of bone samples were removed by compressed air. This process was repeated 5 times until the soft tissue of the bone was completely removed. The samples were washed with

ethanol and dehydrated 24 hr in a desiccator. In order to have a conductive surface the dried bone samples were sputtered with gold. To analyse the organic material without damaging the bone-implant interface after the push-out test, the samples received a special treatment in the Justus-Liebig-Universität Gießen, Germany. The bone-implant samples were initially washed with a 0.1 M sodium phosphate buffer and fixed for 24 hr with 2.5% glutaraldehyde + 1% sucrose. The samples were again washed in 0.1 M sodium phosphate. Finally, the samples were dehydrated in ethanol until the critical drying point was reached with a CPD7501 (Thermo VG Scientific). Metallic samples were ultrasonically cleaned with ethanol for 15 min and then for 5 min in distilled water. Finally, the samples were completely dried.

3. Results and Discussion

3.1 Post-operative biomechanical push-out tests

The implants were located in the distal femoral metaphysis. μ-CT analysis in Figure 6 shows that the implants were surrounded by cancellous bone which has plate-shape structure. The implantation was carried out manually, consequently the implants were found at different position of the distal femoral metaphysis. These positions differed in the amount of cancellous bone surrounding the implants. Additionally, the manual implantation results in misalignment of the implant. During the operation, the top side of the implant was positioned in the cortical bone side (outer darkest area on Figure 6a and b) but some implants were positioned below the cortical bone side. That permits that cortical bone grows and covers the top of the implant.

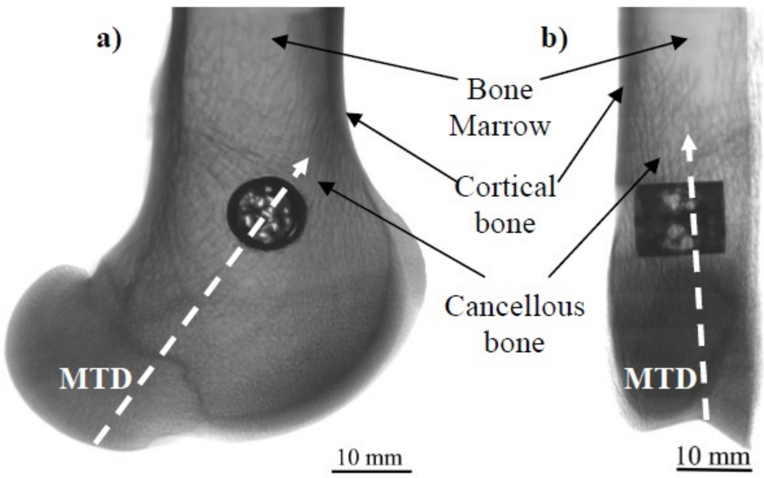

Figure 6: μ-CT images of the ovine distal femoral metaphysis with implants a) frontal view, b) lateral view

The push-out test results exhibit no considerable difference between the coated and uncoated implants (Figure 7). The coated samples show a push-out force of 1419±494 N and an absorbed energy of 3993±2274 mJ while the uncoated samples show similar values with a push-out force of 1362±529 N and an absorbed energy of 3614±1556 mJ. Two control samples of implants in cadaveric sheep bone were tested in push-out testing since all the sheep survived the surgical intervention of the implantation. Both control samples exhibited very low push-out loads in the range of 50N. The evolution of push-out loads can be attributed to bone ingrowth into the cellular structure and osseointegration of the implant during the time it was exposed to the living organism. However, earlier tests carried out on implants in the femoral metaphysis of rats revealed a factor of about 3-4 between control samples (rats which died during the implantation surgery) and samples extracted after 30 days in the living body.

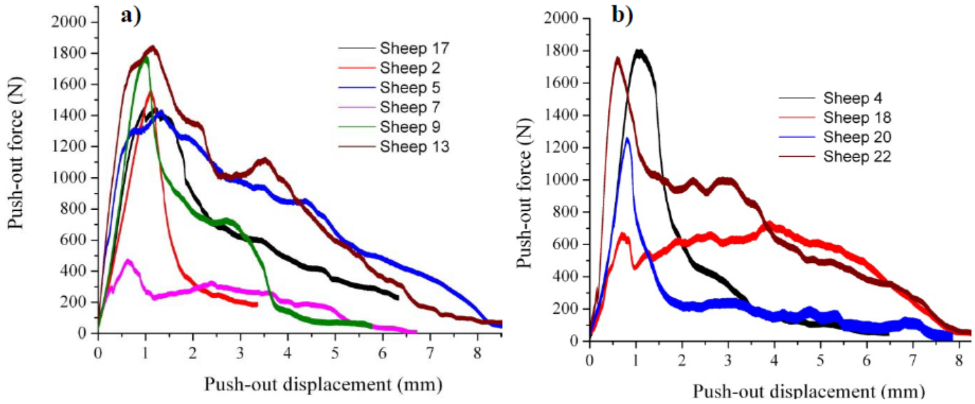

Figure 7: Push-out behaviour of the a) coated ovine implants, b) uncoated ovine implants

Coated and uncoated implants exhibit similar push-out values. However, both groups of implants show two push-out curves with very low values. Push-out result of sheep #7 (coated implants) and of sheep #18 (uncoated implants) reveal very low values. A possible explanation of these low values can be the age of these two animals. At the euthanasia, sheep #7 was 95 months old and sheep #18 was 86 months old. Both sheep were significantly older than the other sheep and therefore the bone growth activity of the sheep #7 and sheep #18 might be lower. Another explanation may be that the implants of these animals occupied a position in the distal femoral metaphysis with reduced amount of cancellous bone. In this case, less cancellous growth inside the implants is expected and consequently that can reduce the push-out values.

3.2 Histomorphological analysis

After the push-out test, the implants were carefully cut along the length and then the fat and bone marrow were removed in order to examine the bone that grew inside the implants. Stereoscopy analysis shows no cancellous bone inside the implants of the older sheep (Figure 8a) while the implant of a younger sheep shows a high amount of cancellous bone in the holes and from the "cancellous side" to the top of the implant (Figure 8b). The lower side of the implant was in direct contact with the cancellous bone while the upper side was in direct contact with the cortical bone. The soft material found at the "cortical section" of the implant was identified as granulation tissue and rest of cartilaginous tissue (Figure 8).

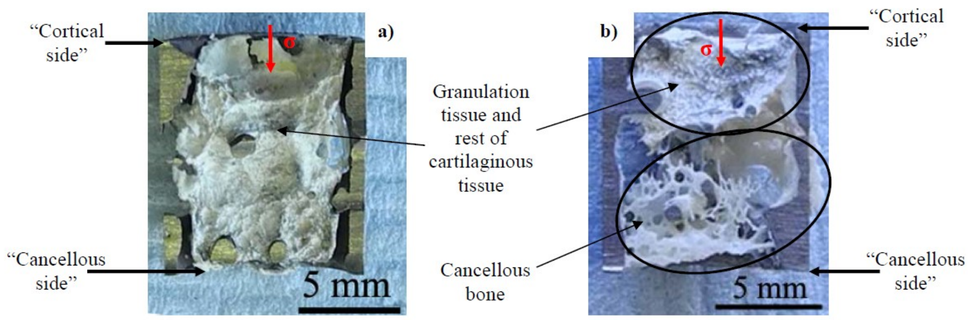

Figure 8: Bone ingrowth in the implants of the sheep a) old sheep #7 (95 months old), b) young sheep #22 (49 months old)

Figure 9 shows the SEM microstructure of the implants of the sheep #5 (58 months old) and sheep #22 (49 months old) and the main directions where the bone grows inside the implants. It was found that not only the bone grows from the "cancellous side" but also from the MTD and through the implant holes. The amount of cancellous bone found in the holes depends highly on their alignment with respect to the MTD. In the case of the implants with one of the holes perfectly aligned to the MTD, the highest amount of cancellous bone ingrowth from this hole through the diameter of the implants was observed. The cancellous bone found in the boundaries of the hole and in the border of the lower side (cancellous side) of the implant was thicker than those found inside the implants. Cancellous bone inside the implants shows a rod-like structure with a trabeculae spacing of 532±232 μm and a trabeculae thickness of 120±50 μm. These values are lower in comparison with those found for the plate-like cancellous bone that surrounded the implants. This indicated that the loads inside the implant are much lower than the loads found at its external surface.

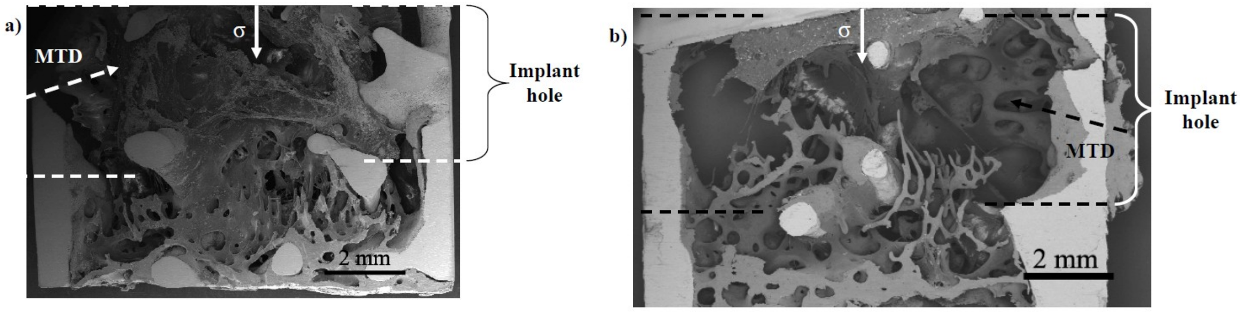

Figure 9: SEM internal micrograph of the implant: a) sheep #5 and b) sheep #22

The external surface of the implants shows a very limited osseointegration with the exception of the areas around the holes (Figure 10a). This limited osseointegration can be explained due to mechanical milling applied to these implants before the operation of the sheep. The consequently reduced roughness is considered prejudicial in the formation of the bone-implant interface. On the other hand, the internal surface of the implant show high osseointegration. In the vicinity of the implants, three forms of tissue were found: 1) cancellous bone, 2) granulation tissue and 3) cartilaginous tissue. Figure 10b shows that the bone with the forms 2 and 3 grows around the surface of the internal struts.

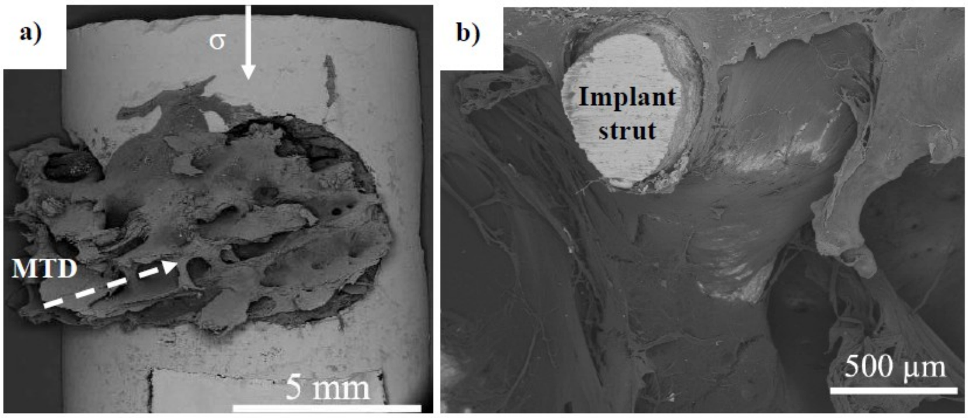

Figure 10: SEM micrograph of the ovine implants after the push-out test: a) external surface of the implant of the sheep #2, b) surface at the internal implant strut of the sheep #22

The failure mode of the cancellous bone showed in Figure 10a was typically found in all the cancellous bones that grow inside the holes of the implants. However, this cancellous bone ingrowth was not always found in all the implants. Cancellous bone deforms by bending and fails by shear at its weakest section and at the regions where the new cancellous bone was not completely mature. The MTD plays an important role in the cancellous bone ingrowth and in the strength of this new cancellous bone. It was noticed that the new cancellous bone around the implants which grew in direction to the MTD were stronger than the bone located at the opposite site of the implants.

4. Conclusions

In this work an open-cell implant on the basis of the titanium alloy Ti-6Al-7Nb was developed which can be used to substitute cancellous bone. Comparative biomechanical push-out tests on samples implanted in the femoral metaphysis of sheep as well as SEM investigations were carried out in order to answer the question whether the application of a biological reaction layer of $CaTiO_3$ is capable of improving the biocompatibility of the alloy in terms of bone ingrowth and osseointegration. The fact that no significant improvement of push-out loads exists between uncoated and coated implants indicates the high level of intrinsic biocompatibility of the Ti-6Al-7Nb alloy. SEM analysis of the samples after push-out testing showed that particularly in the main trabecular direction of the surrounding bone a remarkable amount of trabecular bone has grown inside the cellular structure and along the metallic cell struts of the implant. It was further found that surface condition of the implant plays an important role. While good bone adhesion was observed on surfaced treated with a pickling process after casting the machined outer surfaces of the implants showed less adhering bone after the push-out tests.

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