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Journal of the European Ceramic Society 32 (2012) 2607–2612

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Direct observation of bone coherence with dental implants

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Available online 18 March 2012

Abstract

A newly developed gentle ion beam polishing technique was established for preparing of cross sections of dental implants feasible for high resolution scanning electron microscope investigation. This approach was applied to investigate the interfacial microstructure between newly formed bone and dental implants with modified surfaces extracted after *in vivo* test in adult miniature pigs. The results obtained so far reveal that it has become possible to analyze the bone coherence to implants besides measuring the bone coverage. The amount and density of the mineralized extra cellular matrix has found to be different in different sub-microscopic regions around the implant. From our observations, it can be seen that new bone grows from the existing bone and advances towards the implant surface by in growth mechanism. The images also reveal that new bone is formed directly at the implant surface; we propose a deposition mechanism to explain this. Eventually the in grown and the deposited bone connect to give a good anchorage of the implant. This achievement bears implication for understanding osseointegration at microscopic level.

Keywords: Interfaces; Electron microscopy; Biomedical applications; Osseointegration

1. Introduction

Endo osseous implants are artificial tooth roots used for the treatment of missed teeth. The discovery of titanium screws as dental implants by Brånemark revolutionized the oral rehabilitation therapy.[1] Titanium dental implants allow the growth of osseous tissue into its close proximity, which enables a stable anchorage. This phenomenon is described as osseointegration.[2] The success of oral implant osseointegration is dependent on the surface properties of the implants, *e.g.* the thin surface oxide layer that is always unavoidably formed when titanium is exposed to air. In order to design implants with enhanced properties, for example faster healing and increased initial stability, a careful understanding of the mechanism of osseointegration in the vicinity of the interface between dental implants and hard tissues is necessary.

The dental implant healing process is initiated by the surgical trauma taking place during opening of soft tissue and drilling in the host bone. Blood immediately gets in contact with the surface followed by different cells involved in the inflammatory response. Proteins secreted by the cells can affect inflammation, bone healing and immune reactions as well as alter the structure and physiochemical properties of the implant surface.[3] The bone tissue formation then continues with a serious of complex processes involving, *e.g.* angiogenesis, formation of new blood vessels from pre-existing vasculature, and bone cell differentiation.

Old bone tissue damaged by drilling undergoes a remodelling process in which osteoclasts first resorb bone followed by osteoblasts forming new bone. These remodelling sites forms morphological structures termed cement lines and have a width ranging between 0.2 and 0.5 μm.[4] Interestingly a layer similar to the cement line has also been observed at the implant surface. Davies suggests that the cement like line at the implant surface is part of *de novo* bone formation or contact osteogenesis, *i.e.* bone formation does not only proceed towards the implant from existing bone (distant osteogenesis) but also grow from the implant.[5,6] The cement line at the implant is formed when

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doi:10.1016/j.jeurceramsoc.2012.02.042

osteogenic cells reach the implant surface, secreting collagen-free proteins, later turning into a mineralized interfacial matrix. In front of the cement line a series of events will form a calcified collagen compartment. Thus, there is a collagen-free calcified tissue layer separating the implant from the collagen compartment of bone. Several studies have found osteoblasts, osteoid, and mineralized matrix adjacent to the layer on the implants surface,[7] suggesting bone is deposited on and extending outward from the biomaterial. There are also even results claiming 30% faster bone growth extending away from the implant than moving towards the biomaterial. The contact osteogenesis process seems to be dependent on the surface roughness,[8] maybe as a result of increased possibilities for fibrin to attach to the implant surface and assist bone cell migration.[6]

Bone formation at the implant surface could also be a result of bone growth along the implant surface rather than contact osteogenesis extending directly outward from the biomaterial. Schüpbach et al.[9] showed that implant with an oxidized porous surface (Nobel Biocare TiUnite®) had bone formation starting from old bone at the upper part of the thread, continuing along the implant surface, forming a thin rim. The advancing front of the bone forming rims was occupied by both preosteoblasts and osteoblasts.

As the osseointegration process precedes, the space between the implant and the old bone is filled with woven bone, which grows fast but has a random collagen structure. The woven bone is gradually replaced with lamellar bone, with regular (lamellar) collagen structure, giving it stronger mechanical properties.[10]

The bone coverage is one of the critical parameters used in the histological study to analyze the quality of osseointegration. It defines the percentage of dental implant surface covered by newly formed bone, but do not necessarily indicate the type of bonding or quality of bone around the implant. These parameters are best studied with electron microscopes. Even after more than 40 years of successful clinical use of titanium implants, the nature of the interface between the hard tissue and implant has not been extensively evaluated due to technical limitations in specimen preparation. Artefacts arising from mechanical polishing of the interface limit the resolution and prevent a more detailed, quantitative analysis of the interaction between implant surface and hard tissue. In a previous work we have demonstrated that argon ion beam polishing is a successful route to prepare interfaces for precise characterization of the bone/implant interaction by subsequent SEM imaging.[11] In the present study, this approach was used to visualize the implant to bone interface on a microscopic level for oxidized porous surface placed in mini pigs, identifying contact and distant osteogenesis as well as the bone coherence and bone quality on a microscopic level.

2. Materials and methods

2.1. In vivo tests

TiUnite® implants with an oxidized porous surface (Nobel Replace Tapered Groovy RP 4.3 × 13 mm, produced by Nobel Biocare AB) were used in the *in vivo* study using a mini pig model. The choice of pigs was based on the genetic similarities between humans and pigs, where xenotransplantations are possible.[12] Pigs also demonstrate a good likeness with human bone with respect to macro- and microstructure as well as bone composition and remodelling. Housing and handling of the Göttingen mini pigs used in the study was done in accordance with the approved ethical permission. All mini pigs were at least 2 years old, thus having permanent teeth. The mini pigs were anaesthetized and the 2nd premolars on each side of the mandible were extracted. Careful drilling according to the protocol for placement of a Replace Tapered RP 4.3 × 13 mm implant was done in one of the extraction alveolar: drill with tip tapered Ø 2.0 mm, drill tapered NP Ø 3.5 mm, dense bone drill RP Ø 4.3 mm, and finally screw tap RP Ø 4.3 mm, all 13 mm in length.[13] The drill diameter corresponds to the uppermost part of the tapered drills, the exact dimensions for drill hole *vs.* implant is not publicly available. However, the final tapered drill hole is smaller than the implant, thus when the implant is screwed down the threads get in direct contact with the present bone while the valleys do not. Implants were placed in the fresh extraction sockets after removing the roots, a cover screw was inserted on top of the implant and sutures were used to close the open wound. After 4 weeks of healing the animals were sacrificed, the part of the jaw containing the implants and adjacent teeth were cut out and placed in 4% paraformaldehyde for a minimum of 1 week before further evaluation. In total implants were placed in 21 mini pigs whereof one implant from each pig was further analyzed using the ion beam polishing technique.

2.2. Sample preparation of bone/implant interfaces

After fixation in formaldehyde, the jaws were cut longitudinally in the mesio-distal direction through the middle of the implants using an Exakt cutting unit (Exakt, Norderstedt, Germany) equipped with a diamond-coated band saw. The two resulting halves of the original specimen were dehydrated in ascending grades of ethanol and then embedded in a light-curing one-component resin (Technovit 7200 VLC, Heraeus Kulzer, Wehrheim, Germany).

2.3. Argon ion beam polishing

From one half of the original specimen, prismatic blocks with approximate dimensions 1 mm × 5 mm × 5 mm containing a region close to the tip of the implant were cut using a low-speed saw and diamond wafering blades. These blocks were mechanically polished with 400 grit SiC paper using water as the lubricant. It was then fixed on the sample holder of the cross-section polishing apparatus using carbon paint (Conductive Carbon Cement, Plano, Wetzlar, Germany). Argon milling was performed using SM-09010 Cross-section polisher (JEOL, Tokyo, Japan) at accelerating voltages in the range of 4–4.5 kV with beam currents between 70 and 90 μA for 20–24 h. A precut sample block with mechanically polished surfaces is covered with a shield plate, which stops half of the Ar ion beam. Only an approximately 75 μm wide part of the sample protrudes from the cover. This part is slowly milled by the ion beam, leaving

behind a well polished surface at the position of the edge of the shielding plate.

2.4. Microstructure characterization by scanning electron microscopes

Ion beam polished surfaces were studied using a JEOL JSM-7000F field emission scanning electron microscope equipped with an energy dispersive X-ray (EDX) spectrometer (Oxford Instruments, Abingdon, UK). Accelerating voltages in the range of 5–10 kV were used for secondary electron (SE) and backscattered electron (BSE) imaging. EDX spectra were recorded at 10 kV.

3. Results and discussion

3.1. Microstructure of the interface between bone and implant

Usually the polished section includes one or two flanks of the thread and the region in between the flanks. After the ion beam polishing, it is possible to clearly distinguish the hard and soft materials in the back scattered electron mode due to atomic number (Z) contrast, see Fig. 1. The oxide layer on the implant surface, resin and bone can clearly be distinguished under higher magnifications, see Fig. 2a. The oxide layer is characterized by unevenly distributed pores with diameter 1–5 μm. It is also possible to characterize the filling of pores. The pores, especially close to the titanium core, are only partially filled with bone whereas pores near the surface of the implant are almost completely filled with bone, see Fig. 2a. In a false-colour rendered image obtained from the EDX maps, as shown in Fig. 2b, titanium appears light blue, the oxide layer appears in different shades of purple depending on the local concentration of the incorporated phosphorous in the oxide layer, and bone appears yellow-green. The gap between the implant and the resin/bone must have been formed due to dehydration during the sample preparation. This is still an artefact that is hard to avoid. The

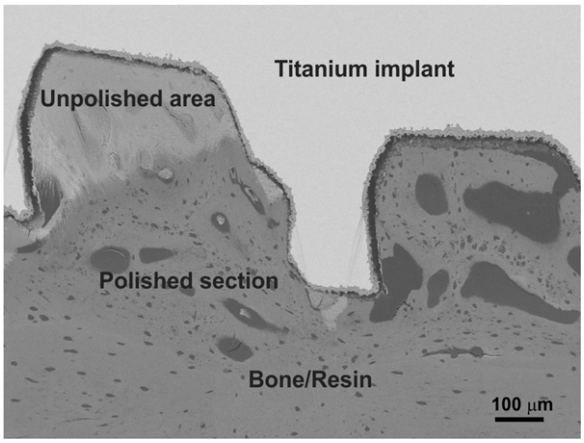

Fig. 1. SEM image in the back scattered mode of the implant after ion beam milling at 4 kV for 24 h. The contrast between hard (implant) and soft (bone) materials is clearly distinguishable as compared to the unpolished area.

close inspections of the interface between bone and implant indicate that the dehydration-induced cracking does not occur exactly between implant and newly formed bone, but inside the newly formed bone immediately close to the bone–implant interface. This evidence reveals that the newly formed bone has good coherence with implant.

The amount and density of bone were found to be different in different regions around the implants. Around the tip of the implant (*i.e.* in the bottom most part of the implant), the bone is found to be less compact as shown in Fig. 3. On the sides of the implant, at lower magnification, the texture of the bone appears different from that on the tip. At these positions, the outer region of the thread show higher bone coverage and higher bone density than the inner area, see Fig. 4a and c. This may be due to the fact that it is easy for the bone to reach to the outer side of the implant when it grows from the already existing jaw bone towards the implant by an in-growth mechanism. The gap between the implant and the bone has always been found to be larger on the inner region of the threads, see Fig. 4b, where the bone has been pulled out from the pores during dehydration.

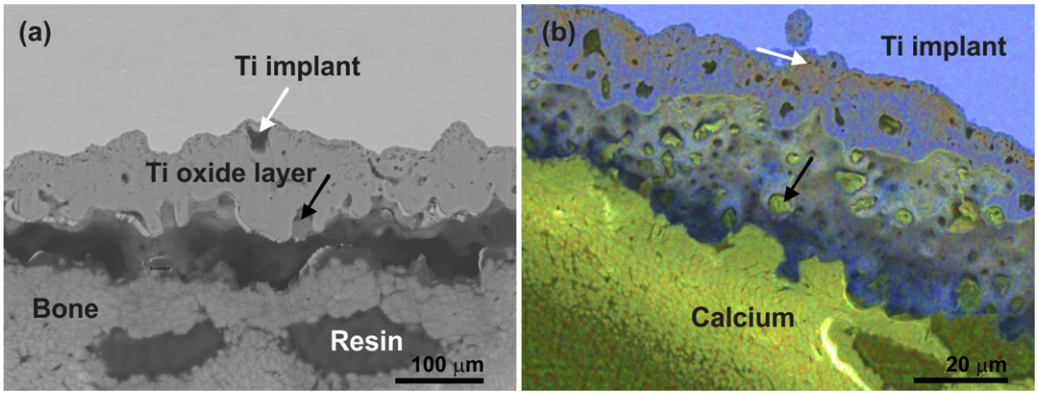

Fig. 2. (a) SEM image of the ion beam polished section of the implant. Ti, oxide, bone and resin/soft tissue can clearly be distinguished due to atomic number contrast. The resin appears black and bone appears dark grey in colour. A pore close to the Ti core is filled with resin only (white arrow) where as the pores away from the Ti core are filled with bone (black arrow). (b) False-colour rendering of a polished section of another implant superimposed to a BSE image. Colours are obtained from the EDX signals (red, phosphorous; green, calcium; blue, titanium). The presence of bone inside pores is confirmed (black arrow). Phosphorous is accumulated at the Ti/oxide interface (white arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

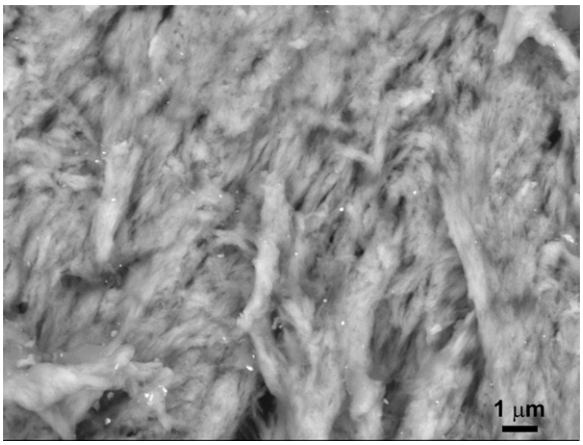

Fig. 3. SEM image of bone closer to the tip of the implant.

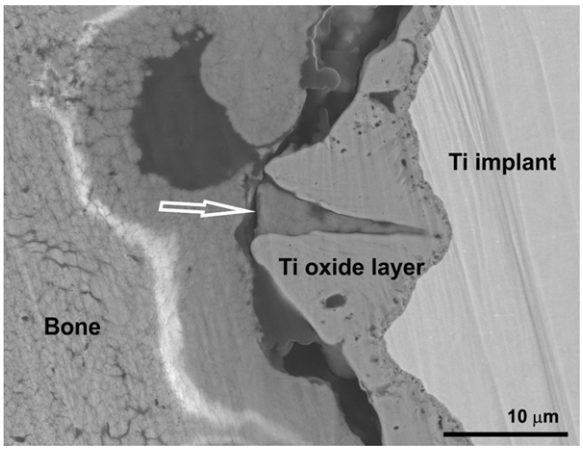

Fig. 5. Filling up of the orifices of one of the large pores of the oxide layer with bone (white arrow).

However in all the implants, the bone integration around the outer side of the flanks survives the dehydration. The fibrous morphology of the tissue pulled out from the surface pores as shown in Fig. 4b seems to indicate that this is incompletely mineralized bone, *i.e.* the bone formed in early stage of osseointegration. From the EDX analyses from this fibrous region, the average atomic percentage ratio of Ca to P was estimated to be 0.95. At this stage the coherence between bone and implant has not established yet. In the outer region of the thread, however, the bone density is quite higher as can be seen in Fig. 4c. This reveals that the bone growth mechanism is different in different regions around the implant.

Two types of bone were observed on the TiUnite® implants, the bone which grows towards the implant and the bone which seems to have deposited on the implant. With the bone in-growth mechanism, newly formed bone grows towards the implant from the already existing one. Such bone growth has been reported in literature as distance osteogenesis.[5,6] In this case, the orifices of large pores of the porous oxide layer are filled with extra cellular matrix, see Fig. 5. The bone density around the immediate vicinity of the implant thread remains the same. The old bone or the already existing jaw bone appears to be denser than that of the newly formed one, see Fig. 6. Atomic percentage of carbon, oxygen, phosphorus and calcium was estimated from the EDX spectra to be 24.40, 54.81, 8.23, 12.56 respectively for the old bone and 31.80, 49.29, 8.05, 10.86 for the new bone. The mineralized extracellular matrix and osteocyte lacunae can clearly be distinguished by atomic number contrast. The highly mineralized cement line matrix between the old bone and the new bone is also seen in some of the polished sections, see Fig. 6b. It is believed that the old bone provides the osteogenic cells that lay down the matrix to encroach the implant. Osseointegration is the result of modelling and remodelling of bone tissue that occurs after implant placement. The ion beam polishing technique enabled us to clearly visualize bone remodelling.

With the bone deposition mechanism, a dense layer of bone is formed immediately on the implant, see Fig. 7a. The quality of this layer of bone is very different from that of the in-grown bone. The in-grown bone shows osteocyte lacunae while the deposited bone looks as if it was formed from precipitation mechanism. The deposited bone is mainly seen on the outer side of the thread and if the implant had additional perforations on it. The deposited bone and the in-grown bone merge together in the later stage of osseointegration, which makes sure that the implant is tightly fixed on the jaw bone. This type of denser bone appears to have good coherence with the implant, see Fig. 7b. Bone coherence was found

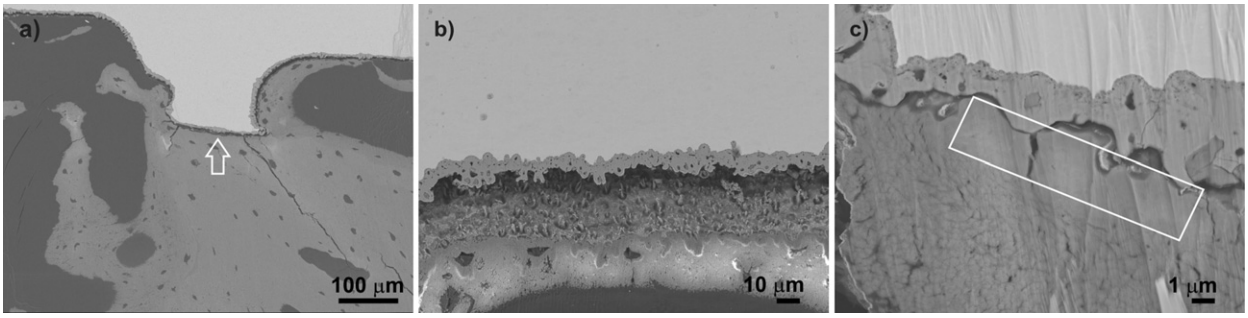

Fig. 4. (a) Bone growing towards the implant. The outer region of the flank is fixed more rigidly by bone (white arrow) than the inner region (b) where the gap between the implant and the bone is always more. This gap comprises fibrous type of bone. (c) A closer look on the outer region of the thread shows highly compact bone than in the inner region (white rectangle).

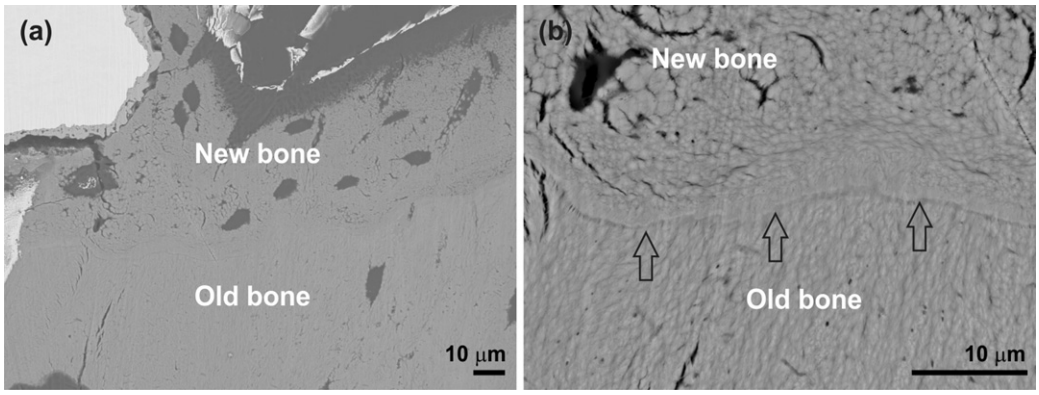

Fig. 6. (a) New bone growing towards the implant is clear from the difference in density from the old bone which appears highly dense. (b) At higher magnification, the cement line matrix which separates old and new bone (black arrow) becomes clear.

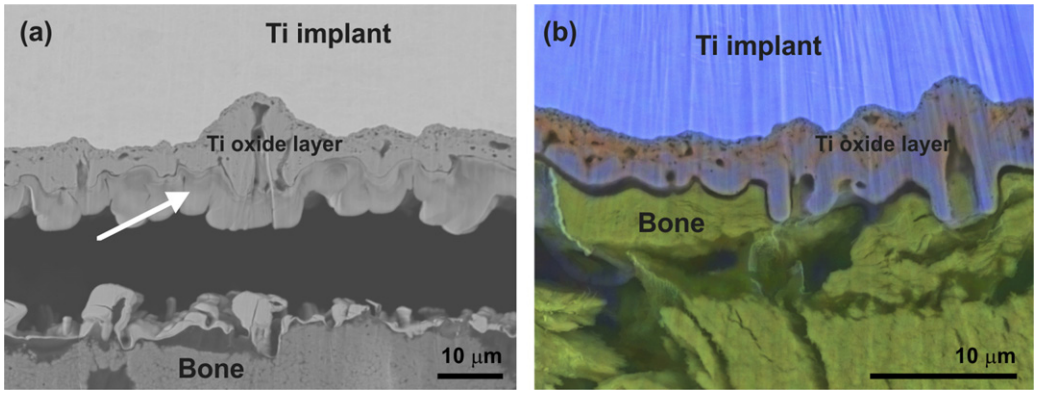

Fig. 7. (a) Bone deposited on an uncoated implant (white arrow). (b) Bone coherent with the implant surface.

more often in the outer region of the thread than in the inner region.

4. Conclusions

Ion beam polishing enables precise characterization of the microstructure of bone–implant interface by scanning electron microscopes. Besides bone coverage the bone coherence on implant can be judged. It was found that the bone coverage and coherence is higher close to the thread of implant than that in the inner regions between threads. Two types of bone growth mechanisms were verified, namely bone in-growth and bone deposition. These observations open up new possibility for understanding of the osseointegration mechanism in more details.

Acknowledgements

This work was supported by the Swedish Governmental Agency for Innovation Systems (Vinnova) and the Swedish Research Council (VR) through the Berzelii center EXSELENT on porous materials. The support of the Knut and Alice Wallenberg foundation for the purchase of electron microscopes used in this study is gratefully acknowledged. We thank Professor Osamu Terasaki for valuable discussions.

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