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COMPARATIVE ANIMAL STUDY ON HARD TISSUE INTEGRATION AND BONE FORMATION OF DIFFERENT NOBEL BIOCARE IMPLANTS

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Dental implantation aims at optimal and long-term hard tissue integration. Beside primary stability, loading time and other factors, *e.g*. the surface of the endosteal part of the implant, is a matter of special importance. In this animal trial, hard tissue integration of two different implant types was studied using radiological, histological and histomorphometric analysis. Two different implants with an oxidized surface (TiUnite; Nobel Biocare AB, Goteborg, Sweden, NobelReplace® Tapered Groovy 4.3 x 10 mm and Replace® Select Tapered 4.3 x 10 mm) were inserted into the right and left mandibles of 10 German domestic pigs between canine and premolar and immediately provided with a ceramic crown. The primary implant stability was determined using resonance frequency analysis. After 70 days, the test animals were killed and specimens were collected for histological and histomorphometric examination. All implants showed good primary stability after surgery. Histological and histomorphometrical analysis revealed no significant differences in the bone apposition. The immediate loading of the different implant types don´t have any negative effects on the bone apposition in the period of 70 days. The long-term effects of immediate loading of these types of implant requires further studies.

Key words: *dental implant, primary stability, hard tissue integration, bone remodeling, osteogenesis, hydroxyapatite*

INTRODUCTION

Titanium (Ti) and its alloys are the most frequently used materials for endosseous implants in odontology and orthopedy due to a high degree of biocompatibility and good mechanical properties. The success in dental and orthopedic implantology greatly depends on the biocompatibility, chemistry and microtopography of the metal implant surface, since these properties contribute to its osteoconductivity.

The macroporous surface is beneficial to improve the fixation of implants by the ingrowth of bone into the coating at early postimplantation periods (1, 2). In order to achieve greater peri-implant bone formation, the surface must promote osteogenesis while delaying bone resorption, ultimately producing more bone volume prior to remodeling (3). The characteristics of the implant surface is particularly important in the early phase of peri-implant bone healing, and the bone tissue microstructure is mainly related to the remodeling processes which take place around dental implants (4). It has been suggested that microroughness, produced by blasting and/or acid etching, gives an increase in the implant surface area and enhances biomechanical bonding by optimizing the biological response of the bone and micromechanical interlocking (5). Rougher implant surfaces have been reported to have higher bone-to-implant contact percentages than machined surfaces (6). Titanium implant surfaces with rough microtopographies exhibit increased pullout strength *in vivo* (7), suggesting increased bone-to-implant contact.

The direct adhesion is the main factor responsible for the relation between cellular response and implant surface chemistry and morphology. The term adhesion in the biomaterial domain covers different phenomena. The protein adsorption is well known to be the first event that takes place after contact with body fluids and is influenced by physicochemical characteristics of the materials. The proliferation and differentiation of osteoblastic cells have been shown to be sensitive to surface microarchitecture (8).

Several studies indicated that the oxide structure and porous morphology of the titanium surface are responsible for apatite deposition (9). In order to obtain an improvement in the osteointegration phenomenon, the implant surfaces have to show an ideal micromorphology, that allows the perfect adaptation of bone cells. The bone fundamental substance is able to adapt to surface irregularities between 1.0 and 100.0 μm (10). To the actual study we used an implant with intentional change in surface roughness. The micrometer levels of the pores was between 1 and 10 μm.

Because of preparation for a long-term study of the bone response, the aim of this study was the investigation of the influence of the surface topography of TiUnite® (Nobel Biocare,

Gotenborg Sweden) on the healing process of bone adjacent titanium implants *in vivo* for 2 months.

MATERIAL AND METHODS

In this study two different NobelBiocare implants with an oxidized surface (TiUnite; Nobel Biocare AB, Goteborg, Sweden, NobelReplace® Tapered Groovy 4.3 x 10 mm and Replace® Select Tapered 4.3 x 10 mm) were inserted into the right and left mandibles of pigs (Sus scrofa domestica) according to a uniform protocol, respectively. The surfaces of the implants were produced by anodic oxidation and differ from each other. NobelReplace® Tapered Groovy has a thread and a shoulder with grooves, whereas Replace® Select Tapered has a smooth surface on these regions. The titanium porous oxide (TPO) was analyzed under S.E.M (Scanning Electron Microscopy – JEOL JSM-35) to observe the surface morphology of the implants (*Fig. 1*).

Eight 15 to 16 months old animals with an average weight of 80 kg were used. The protocol of this study was approved by the Ethical Committee for Animal Research LVL MV/TSD/7221.3-1.1-037/05. Mandibular premolars P2-P4 were extracted bilaterally. After 3 months of healing, the screws titanium implants were fixed between the canines and molars (a total of two implants per animal). All implants were inserted by a standard surgical technique of an implant system. The implants shoulders were placed at the ridge crest level.

All implantations were carried out by one person to reduce the application fault and the implants were immediately provided with a ceramic crown (Ceramic Copping Easy Abutment™, NobelBiocare) followed by the Resonance Frequency Analysis (RFA) of each implant to analyze and evaluate the implant-bone stability.

The surgical procedures in the animals were performed under general anesthesia (i.v. injection into v. auricularis caudalis) of ketamine hydrochloride 150-200 mg (Ketamin, Belapharm, Vercha, Germany), droperidole 4.0-7.0 mg (Droleptan, Janssen Pharmaceutical, Oxon, UK) and Faustan 4.0 mg (Temmler Pharma, Marburg, Germany). In addition, approximately 1-2 ml of 2% Xylocaine®/Epinephrine 1:50.000 was injected at the surgical site to reduce bleeding. No plaque control program was used in this study.

To assess the repair process and bone remodeling during the healing period intravenous fluorochrome substances injections (*Table 1*) were used. After 70 days postoperatively, the animals were killed with an intracardiac injection of a high dose (5 mg/kg) of pentobarbital natrium (Eutha 77, Essex-Pharma, Munchen, Germany). The implants/bone complexes were removed, fixed in 4% buffered formalin and embedded with Technovit 9100 New® (Kulzer & Co, Wehrhein, Germany). The histological preparations were processed in agreement with the technique established by Gedrange *et al.* (11).

Histomorphometric analysis

In order to improve assessment of bone apposition, the sections were stained by Masson-Goldner yielding green-coloured bone and connective tissue. After staining the implant longitudinal sections were photographed using an Olympus BX 61 microscope and a Colour View 12 digital camera (Soft Imaging Systems Ltd.) with 4X lens magnification and analysis® 3.0 software.

The menu item "Multiple Image Alignment" in connection with the motor-driven scanning table (Marzhauser Comp.) enabled the take of 2 x 7 single images along the implant longitudinal axis and the composition of an overview screen. Subsequently, bone apposition was measured in relation to implant surface. Means and standard deviations were calculated through the bone apposition rates of the individual sections and summarized for the different surface coatings.

The standard t-test was used to calculate the quantity of new bone formed around the TiUnite® surface of NobelReplace® Tapered Groovy and Replace® Select Tapered. This t-test was also used to calculate the significance of data differences ($p \leq 0.05$) between bone and osteoid matrix deposited in the pre-existent space among implant spirals.

Table 1. Polyfluorochrome Sequencial Labeling.

Time	Substance	Doses—injection (mg/kg)
Day 14	Oxytetracycline	10 mg/kg (yellow)
Day 28	Calcein	05 mg/kg (light Green)
Day 56	Xylenol orange	60 mg/kg (Orange)
Day 70	Exitus	----------

RESULTS

The superficial analysis under SEM (*Fig. 1*) of differents implants patterns showed the morphological surface from TPO from Nobel Biocare.

Histological evaluation

The light microscopy evaluation revealed no remarkable differences in bone formation using NobelReplace® Tapered Groovy (RTG) and Replace® Select Tapered (RST) implants compared to TPO implants (*Figs. 2* and *4*). The ossification within and immediately adjacent to the threaded implant surface was the predominant observation using these two implants.

The interface of the both implants demonstrated the existence of osteoid tissue matrix sedimented in the immediate area between bone and implant (*Fig. 3* and *5*). The RST implant samples showed bone lamels parallel to the spiral surface beyond points with bone contacts between the surface and the new tissue (*Fig. 3*). In the RTG implants we noted the growth of mature bone tissue in the spaces among the spirals (*Fig. 4*). The grooves situated in the inferior side of these implants spirals did not show collagen fibers and bone lamels after the 70 days of tissue reparation period (*Fig. 4* and *5*). Otherwise, the osteoid matrix was found in these grooves (*Fig. 5*).

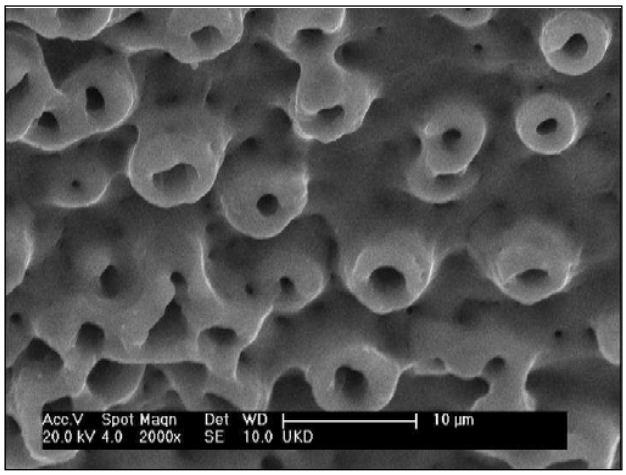

Fig. 1. Photomicrografy under SEM. TPO surface. Bar -10 µm.

Fig. 2. Photomicrografy under light microscopy. Replace® Select Tapered with mineralized new bone formation Masson Goldner. Bar - 1 mm.

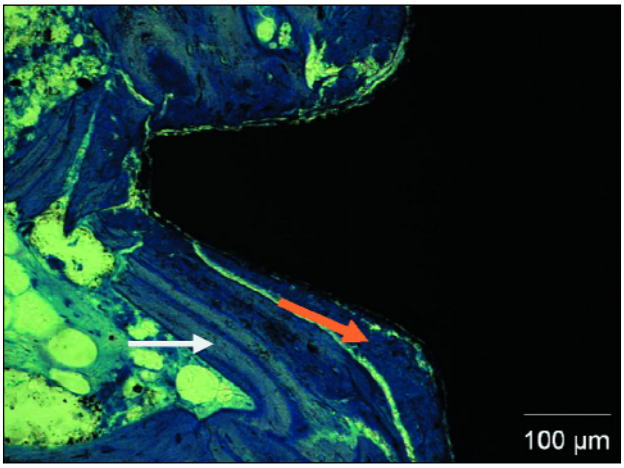

Fig. 3. Photomicrografy under light microscopy. Replace® Select Tapered, bone interface with lamellar bone (white arrow) and osteoid matrix (orange arrow) in contact with implant surface. Toluidine Blue. Bar - 100 µm.

Histomorphometric evaluation

The result from the histomorphometric evaluation is presented in *Fig. 6*. There were no significant differences in the bone and osteoid matrix formation within and immediately outside the thread area between the two different implant morphologies of the TPO surface. According to the *t-test,* the two tested implants demonstrated similar bone and osteoid matrix deposition. The differences between the two implants relating to bone matrix (RST–60.23% and RTG–60.15%) and osteoid matrix (RST–3.56% and RTG–3.41%) were considered to be not significant, because the P values were less than 0.05.

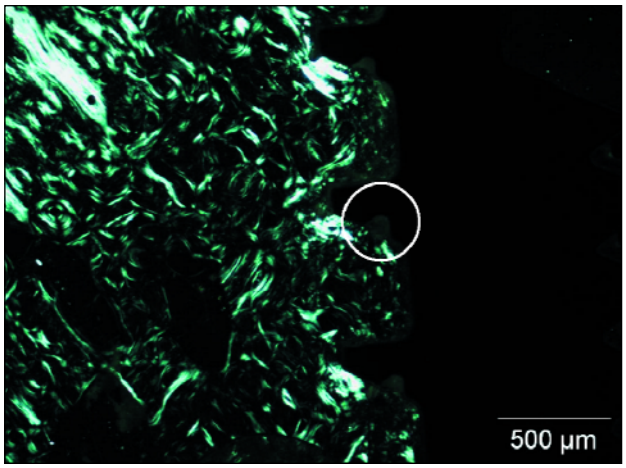

Fig. 4. Photomicrografy under polarized microscopy. NobelReplace® Tapered Groovy. Bone interface with collagen fibers type II. In the Groovy region it was not noted (white circle) collagen fibers fullfiled. Bar - 500 µm.

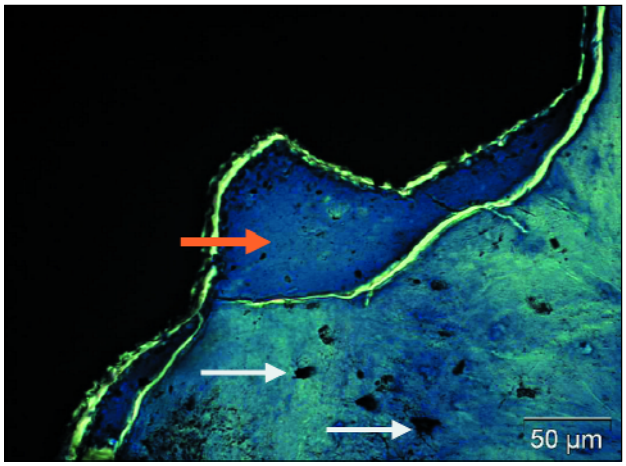

Fig. 5. Photomicrografy under light microscopy. NobelReplace® Tapered Groovy. Collar grooves region fulled with osteoid matrix (orange arrow) and new bone formatted with osteocytes cells (white arrows). Toluidine blue. Bar - 50 µm.

Fluorescence evaluation

The measurement of the bone fluorescence showed new bone formation onto the implant threads. The fluorescent markers indicated slow new bone formation as observed by narrow oxytetracycline yellow (week 2), calcein green labels (week 4) and xylenol orange (week 8), and limited bone formation among the labels. The fluorescence microscopy evaluation suggested minimal differences in the bone formation and the bone–implant contact of the two tested implants.

DISCUSSION

The implant surfaces are modified with the aim of obtaining the best response in the tissue deposition (12). Wennerberg *et al.* (13) proposed that the quantity of the bone in contact with implant surfaces depends on the superficial roughness. Previous researches had shown that the surface characteristics were important in influencing the bone-implant contact. In a review

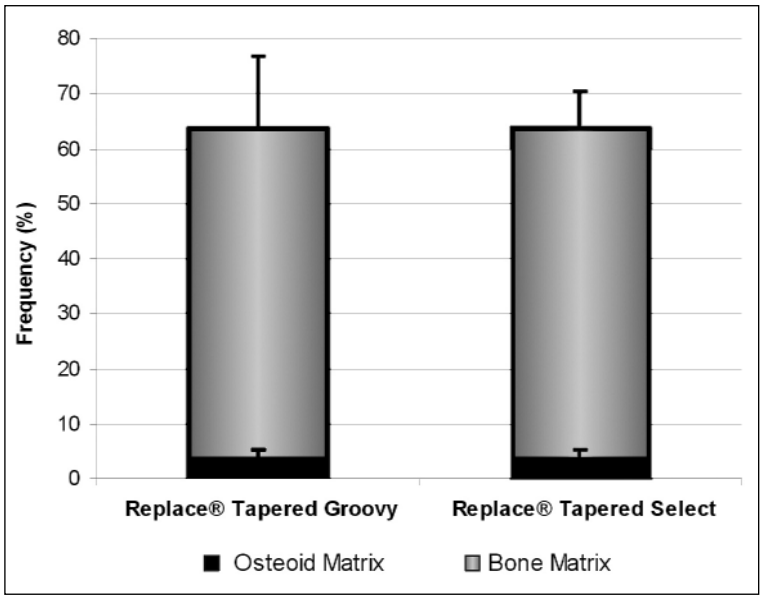

Fig. 6. Representative histomorphometry (t-test) of osteoid matrix deposited on the implants surface from NobelReplace® Tapered Groovy (3.41%) and Replace® Tapered Select (3.56%) and bone matrix deposited on the implants surface from NobelReplace® Tapered Groovy (60.15%) and Replace® Tapered Select (60.23%) obtained by light microscopy. Differences were considered no significant when *P* values were less than 0.05.

about the effects of implant surface topography, Brunette (8) emphasized the roughness and carried out an important role in favour of the percental bone over growth of the implant surfaces. Cochran *et al*. (14) verified that implants containing a uniform surface roughness have a bigger bone/implant contact compared to those with irregular roughness. Various treatment methods were used in order to get more homogeneous surfaces and to permit a better adaptation from the bone cells to the implants.

The characteristic and properties of the TPO surface utilized in this study have been published (15) and are summarized as follows: 1) The TPO consisted of an essentially pure partly crystalline TiO_2 oxide. The oxide thickness increased continuously from 1-2 µm at the upper part to 7-10 µm at the apical region of the implant; 2) The surface roughness and area increased continuously from the flange to the apical part of the implant, where surface roughness was 1.2 µm and the area increase compared with an ideal flat surface was 95%; 3) The surface showed a rough surface topography without sharp features; and 4) The surface (apical portion) contained numerous open pores, with orifices predominantly in the range of 1-2 µm (16). The implants superficial morphology associated to the type of bone tissue used in the present study could have an influence positible on the research results.

The majority of the scientific researches relate that the contact rate between implant and bone depends more on the superficial condition than the material composition. Several studies tended to modifications in the macrostructure as well as in the microstructure of smooth titanium implants. On smooth surfaces the cells attach and proliferate but they exhibit relatively low expression of differentiation (9). Boyan *et al.* (17) analyzed in a literature review the different probable reasons of which the superficial roughness can influence the cell response to the implant. On rough surfaces, the mesenchymal cells form focal connections are able to cross the space among surface peaks. This can result in the expression of specific phenotypes, what is favorable to osteogenesis. Besides, rough surfaces can facilitate blood flow, maybe another reason for the improved osteogenesis. According to Boyan *et al*. (9), when osteoblasts are grown on surfaces with microarchitectures cell attachment and proliferation were reduced, the differentiation was enhanced and the cells increase the production of factors, like TGF β1, that stimulates osteogenesis.

A lot of studies have shown that crystallinity plays a role for apatite formation on TPO surfaces (18). According to Xiropaidis *et al.* (16) the apatite is formed at the crystalline TPO surface *in vivo*. It will then resemble the hydroxyapatite surface with respect to the chemical composition. Furthermore, the authors noted that phosphates are partially covering the TPO surface (15), and that similar phosphate groups exist at the hydroxyapatite surface. Such phosphates may also play a role in apatite formation. Zechner *et al*. (19) demonstrated that hydroxyapatite coated and TPO implants exhibited similar and significantly higher bone-implant contact levels compared to turned titanium implants in a mini-pig posterior mandible model. Apparently, the data obtained in the present study demonstrated the formation of a mature and mineralized cancellous bone around the installed implants. Otherwise, the region did not show the structure of mature bone tissue, characterizing the necessity of a longer reparation period in order to occur the inorganic component deposition.

The percentage data collected of bone matrix formation in this study indicate that the implants placed in the posterior mandible of the pigs were placed in type II bone. On this type of bone the different implants tested do not seem to have influence on the overall bone metabolic activity in a surface dependent fashion. Surface modifications may be more critical in less favorable situations when the bone density is lower (16). Several oral implant design advances have been suggested to overcome poor bone quality, an impediment for successful implant treatment. According to Wikesjo *et al*. (20) the TPO surface possesses a considerable osteoconductive potential promoting a high level of implant osteointegration in Type IV bone in the posterior maxilla.

CONCLUSION

Regardless of surface modifications, a high degree of bone implant formation was found in the both studied titanium implants. The period of reparation was not enough for filling the preexistent grooves of RTG implant models with mature bone tissue. The TPO surface was considered to be efficient in the osteoconduction process of cancellous bones in the studied animals.

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