

Abrahamsson I, Berglundh T, Linder E. Early bone formation adjacent to rough and turned endosseous implant surfaces: An experimental study in the dog. Clin Oral Impl Res 2004; 15(4): 381-392. (23 Refs)

Purpose: To evaluate the rate and degree of osseointegration on two implant surfaces—turned/machined (T) and sand blasted with large grit and acid etched (SLA).

Materials and Methods: For a prior study by the same authors, a titanium implant similar in shape to the Straumann ITI® solid screw implant was designed with a pitch distance of 1.25 mm and a 0.40 mm trough between the threads to form a wound chamber for microscopic evaluation. The experimental device had either turned/machined (T) or sand blasted with large grit and acid etched (SLA) surface. Following 3 months of healing after extraction of mandibular pre-molars, two of each surface type were placed in the mandible of 20 Labrador dogs. The implants were placed at various times to allow for the sacrifice of the animals and biopsies to be obtained at 2 hours, 4 days, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks and 12 weeks post-insertion. Biopsy material was prepared into decalcified and non-decalcified sections for examination. In the non-decalcified sections, the implant shoulder, the bone crest, and the most coronal bone-to-implant contact were identified. The proportion of mineralized bone in contact with the implant surface was determined. The proportions of woven bone, lamellar bone, residual tissue and bone remnants were also determined. In the decalcified sections, the presence of osteoblasts, fibroblast-like mesenchymal cells, adipocytes, erythrocytes, PMNs, lymphocytes, plasma cells, macrophages, vascular structures and residual tissue was assessed.

Findings and Conclusions: At 2 hours, erythrocytes in a fibrin network were observed. The implant shoulder to bone-implant contact point was 5.1 mm on the T implants and 5.8 mm for the SLA implants. From 2h to 1 week, the trough was 4-5% woven bone, ~40% lamellar bone and ~50-60% non-mineralized tissue. At 4 days, osteoclasts were evident surrounding residual bone and near the cut surface of the bone. Fibroblast-like cells were oriented parallel to the SLA surface. At 1 week, the first sign of bone formation is apparent. On the T surface, the new bone was an extension of the surrounding bone; whereas, on the SLA surface it was appositional from the surrounding bone as well as directly on the implant surface. At 2 weeks, bone formation continued in a similar manner. Week 4 presented evidence of remodeling which continued as evident in biopsy material from week 12. At 6 and 12 weeks, the proportion of lamellar bone adjacent to the SLA was greater than the T surface. At the time of placement, only the pitch areas and the bone were in contact. At 1 week, the bone implant contact is 24.8% for SLA and 13.9% for T implants. At 4 weeks, the bone implant contact is ~65% for the SLA surfaces and seems to remain up to 12 weeks. For the T implants, 36.8% bone implant contact is evident at 12 weeks. In the wound chamber, differences in bone formation for turned/machined (T) and sand blasted with large grit and acid etched (SLA) implants were evident initially. After week 6, wound chambers for both surfaces appear similar; however, the bone-implant contact is greater for SLA surface for the dog model.

Traini T, Degidi M, Caputi S, et al. Collagen fiber orientation in human peri-implant bone around immediately loaded and unloaded titanium dental implants. J Periodontol 2005;76:83-89. (54 Refs)

Purpose: To evaluate in humans collagen fiber orientation in immediately loaded and unloaded dental implants.

Materials and Methods: Ten 3.8x9.5mm dental implants (Xive, Dentsply Friadent, Mannheim, Germany) were placed in 10 human subjects as part of a restorative treatment plan. Patients were edentulous in the mandible and were to be restored using 10 implants per patient in one of two surgical protocols. 5 patients were to receive a full arch immediate provisional prosthesis with loading of the implants that same day. The remaining 5 patients had implants placed using a submerged protocol without loading. One additional implant [Test implant(T)] was placed in each of these 10 patients in the distal most aspect of the mandible, thus all patients received a (10+ 1(T) arrangement). 6 months of healing was allowed before removing with a 5 mm trephine each of the 10 (T) implants for study. Implants and surrounding bone were placed in 10% formalin for histological processing.

Two sections per implant were prepared and observed under circularly polarized light (CPL) at 50x magnification. Eight images were evaluated for each specimen (two for each side of the section). Areas of analysis were grid formatted into pixels and then digitized and stored in a tif format. All image pixels were then converted to grey scale and assigned a value between 0 to 255 (0= black, 255= white). Software image analysis was used to quantitate the area's extension relative to collagen fiber orientation and was expressed in square pixels.

Findings and Conclusions:

Table 1.

Statistical Evaluation of Collagen Fiber Area in Bone Around Dental Implants (mean ± SD)

Group	Collagen Fiber Orientation (square pixel)	
	Longitudinal	Transverse
Loaded (N = 40)	13,676 ± 2,232*	45,481 ± 3,037*
Unloaded (N = 40)	89,073 ± 1,960*	32,174 ± 2,554*
P value	<0.001	<0.001

* Significantly different between loaded and unloaded groups in both longitudinal and transverse orientation (unpaired t test: P < 0.05).

Transverse collagen fibers were more abundant under the lower flank of the loaded implant threads, while a more longitudinal arrangement was seen in this area with unloaded implants. Lamellar bone with osteons and vessels were viewed in close proximity to the implant surface. Loaded implants had a 1:3 ratio of longitudinal collagen to transverse collagen arrangement, while unloaded implants had almost a 3:1 (2.76:1) arrangement, respectively. Thus it appears that a magnitude of load forces must be responsible for this adaptive, remodeling of collagen observed in this study.

Sykaras N, Lacopino AM, Triplett RG, Marker VA. Effect of recombinant human bone morphogenetic protein-2 on the osseointegration of dental implants: a biomechanics study. Clin Oral Invest 2004;8:196-205.

Purpose: To evaluate biomechanically the effect of recombinant human bone morphogenetic protein (rhBMP-2) and correlate it with periotest and radiographic measurements.

Materials and Methods: 6 male adult dogs were used in the study. All mandibular premolars were extracted bilaterally using high speed carbide burs. After a healing period of 8 weeks, radiographs were taken to evaluate the bone quality and quantity and each dog was scheduled to receive 6 implants (3 control in one side, and 3 experimental in the contralateral side) and followed for 4, 8 and 12 weeks. Side and sites were alternatively assigned for each dog. Titanium, plasma-sprayed, hollow cylinder implants 3.5mm in diameter and 8mm in insertion depth were implanted as control implants, whereas in the contralateral sites the hollow chambers of the implants were filled with a solution of rhBMP-2 soaked on absorbable collagen sponges. 12 implants were scheduled for the 12 week healing period was inserted in all 6 dogs at baseline. 4 and 8 weeks after initial implant surgery, the same surgical procedure was followed for the implantation of additional implants in all dog, 12 each for the 4- and 8-week healing periods. Periotest values (PTV) were recorded for each implant at the time of euthanasia and before specimen collection. 3 recordings were collected for each implant, and the average value was designed as the PTV for that implant. The specimens were collected for pullout biomechanical testing and radiographic evaluation of bone-implant contact level.

Findings and Conclusions: All dogs healed without any major complications and a few rhBMP-2 implant sites exhibited some minor inflammatory reaction. At the time of death, 10 implants were lost and 4 were associated with large bone defects. The mean pullout forces at 4 and 8 weeks had a tendency to be higher for controls than for rhBMP-2 implants, whereas at 12 weeks the latter exhibited a mean peak force of 795N, vs. 618N for controls. The increase in peak force as a function of time was significant for implants combined with rhBMP-2 but not for controls, and the slope of linear regression for the rhBMP-2 implants was more than three times greater than the slope for the controls. When the force values and toughness values were compared with the corresponding displacements, a higher correlation was observed for the rhBMP-2 implants than for controls. Stiffness value increased over time at weekly rates of 62.7N/mm for the rhBMP-2 implants and 17.1N/mm for the controls, providing evidence of a statistical difference in stiffness values for the rhBMP-2 implants between all time periods but not for the controls. When bone-implant contact was radiographically calculated as percentage of the total implant length, no statistical difference was found between or within the 2 groups at any time. Periotest value comparisons between and within the 2 groups at all time periods provided no evidence of statistically significant differences.

The results from the pullout test support the potential role of rhBMP-2 in clinical applications by promoting a biomechanically mature interface at 12 weeks. However, radiographic and periotest assessment of the bone-implant interface did not provide evidence of the differences observed with biomechanical testing.

Maria Bächle, Ralf J. Kohal. A systematic review of the influence of different titanium surfaces on proliferation, differentiation and protein synthesis of osteoblast-like MG63 cells. Clinical Oral Implants research 2004; 15 (6): 683-692.

Purpose: To evaluate and summarize the medical and or dental literature aiming to assess on which kind of titanium surface structure the osteoblast-like osteosarcoma cells MG63 show the best proliferation and differentiation rate, and the best protein synthesis.

Materials and Methods: A systematic search was done using the following on-line databases: 1)PubMed, 2) Web of Science, 3) Cochrane Library, and 4) International Poster Journal). Handsearch was also done in selected journals, which are not included in Pubmed by examination of the bibliographies of the identified articles. The subject terms used for the search were: osteoblasts, titanium, osteoblast and titanium, MG63 AND titanium and osteoblast-like cells AND titanium-surfaces.

Findings and Conclusions: 348 references were found using the systematic search. Nine articles referring to nine different studies were relevant. Additionally 8 less relevant articles were identified. While the average roughness values of the 'modern' microrough implant surfaces range from 0.9 to 1.4 microns the MG63 cells in the reviewed in vitro experiments favored rough surfaces with average roughness values in the range of 4–5 microns. It was also suggested that there are no differences in cell proliferation and differentiation on surfaces treated by blasting and etching. The surfaces and culture conditions varied widely. Therefore the recommendation of a particular particle size is very difficult. The described in vitro studies support the hypothesis that surface roughness may have direct effects on osteoblast migration, attachment, proliferation, and differentiation. The use of human cell lines such as MG63 is providing a useful tool both to investigate the effects of biomaterials and to understand the mechanisms of cell response. To comprehend the mechanisms of bone formation at the implant surface, the effects of the material on the surrounding cells and on the profile of cytokines, growth factors and other local mediators must be understood.

Hagewald S, Pischon N et al. Effects of enamel matrix derivatives on proliferation and differentiation of primary osteoblasts. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontics 2004; (Aug)92(2):243-9

Purpose: to investigate the effect of EMD on proliferation, protein synthesis, and mineralization potential of cultured primary osteoblasts.

Materials and Methods: Osteoblast cells were enzymatically isolated from 18-day-old mouse calvaria. Calvaria were dissected aseptically and digested on 0.2% collagenase. The released cells were harvested and washed in growth medium. All monolayer cultures were maintained in a standard medium. Prior to the experiment, the osteoblastic phenotype of the cells confirmed by measuring alkaline phosphatase activity and formation of mineralized nodules in vitro. Cell metabolism assay: one thousand cells were cultured in each well of 96-well dishes in standard medium and allowed to adhere overnight. On the following day, media were replaced with experimental media consisting of DMEM containing 2% FCS control or DMEM containing 2% FCS and various concentrations of the test substance EMD (2-100 µg/ml). Measurement of the nucleic acid synthesis: Nucleic acid synthesis in cells was assessed by the colorimetric immunoassay (BrdU kit). Osteogenic differentiation in organoid culture: For the mineralization assay, calls were grown as a dense cell mass in a 3-dimensional organoid culture system. Mineralization was estimated by calcium accumulation in the cultures and by activity of alkaline phosphatase (ALP). After 21 days of EMD treatment, cells were washed with PBS and homogenized in distilled water. Collagen matrix synthesis: To estimate collagen synthesis in the 21 day organoid culture, radiolabel [³H]-proline was added during the last 2 days to the growth medium. Total protein determinations was used to calculate the [³H]-proline activity. Data analysis: Data were processed with the statistical software package SPSS 10.0. Data were analyzed by 1-way analysis of variance, using Bonferroni's modification for post-hoc testing.

Findings and Conclusions: Following exposure of primary mouse osteoblasts to increasing concentrations of EMD over a 48-hour period, a consistent and reproducible stimulation of metabolic cell activity was observed. BrdU incorporation in proliferating cells was enhanced by EMD treatment compared to controls and was maximal at a concentration of 2µg/ml EMD. The mouse osteoblasts revealed high levels of osteoblastic markers such as ALP activity and calcium accumulation after 14 and 21 days in culture. Under the influence of EMD, significant increase of ALP activity and calcium accumulation in the cultures were found at all concentrations tested after 21 days. The maximal mineralization was observed at 100µg/ml EMD. After 2 days of [³H]-proline exposure in the organoid culture, the uptake of the labelled nucleotide did not significantly change in the presence of EMD when compared to the control. Electron-microscopic examination of the control cultures revealed several layers of osteoblastic cells embedded in a moderately dense collagenous matrix. Cultures treated with EMD showed an increase in the number of large mineralized nodules, indicating an acceleration of mineralization. These mineralized nodules were mainly arranged in clusters surrounded by matrix which appeared as moderately dense as shown in the controls. These results showed that EMD may play a role in bony wound healing. EMD seems to be meaningful for early proliferation and later differentiation of osteoblasts which is based on promoting mineralization parameters in the organoid culture model.

Botticelli D, Berglundh T, Lindhe J. Hard-tissue alterations following immediate implant placement in extraction sites. J Clin Periodontol 2004; 31: 820–828.

Purpose: To study dimensional alterations of hard tissues that occurs following tooth extraction and immediate placement of implants.

Materials and Methods: Eighteen healthy subjects (nine female and nine male; mean age, 49.1 years; range, 21–81) providing 21 extraction sockets were included in the study. A full thickness flap was elevated, the tooth extracted, and an implant was placed in the extraction socket. After implant installation, the defect that occurred between the bone walls of the extraction socket and the implant surface was characterized and the following landmarks were identified: S=rim of the implant shoulder, C=top of the bone crest, OC=outer border the bone crest, D=base of the defect.

Following implant installation (i) the vertical distance between the implant shoulder (S) and the bone crest (C), (ii) the width of the gap between the implant surface and the inner side of the bone wall (G) and (iii) the horizontal distance between the implant surface and the outer side of the bone crest (OC) were assessed.

The flaps were subsequently replaced and secured with sutures in such a way that the healing cap of the implant was exposed to the oral environment. After 4 months of healing a re-entry procedure was performed and the clinical measurements were repeated.

Findings and Conclusions: Fifty-two marginal defects exceeding 3mm were present at baseline: 21 at buccal, 17 at lingual/palatal, and 14 at approximal surfaces. At the re-entry eight defects exceeding 3.0mm remained. During the 4 months of healing, the bone walls of the extraction underwent marked change. The horizontal resorption of the buccal bone dimension amounted to about 56%. The corresponding resorption of the lingual/ palatal bone was 30%. The vertical bone crest resorption amounted to 0.3 ± 0.6 mm (buccal), 0.6 ± 1.0 mm (lingual/palatal), 0.2 ± 0.7 mm (mesial), and 0.5 ± 0.9 mm (distal). The marginal gap that occurred between the metal rod and the bone tissue following implant installation in an extraction socket may predictably heal with new bone formation and defect resolution. The current results further document that marginal gaps in buccal and palatal/lingual locations were resolved through new bone formation from the inside of the defects and substantial bone resorption from the outside of the ridge.

Ayukawa Y, Okamura A, Koyano K. Simvastatin promote osteogenesis around titanium implants. Clin Oral Impl Res 2004; 15(3): 346-50.

Purpose: To examine the effect of simvastatin on the promotion of osteogenesis around titanium implants by both histological and histometrical procedures.

Materials and Methods: Ten 30-week-old female rats were used. Under systemic anesthesia, all rats received commercially pure titanium implants (1 mm in diameter, 1.5 mm long, 0.438 μm average surface roughness) in both tibiae. After implantation, the animals were divided into two groups. From the time of implant installation, the experimental group was intraperitoneally administered 10mg/kg of simvastatin daily. The control group received the isotonic saline instead. Thirty days later, all animals were deeply anesthetized, and then perfused with a fixation solution consisting of 0.1 M phosphate-buffered 5% glutaraldehyde and 4% paraformaldehyde. Specimens were further immersed for a week. Afterwards, undecalcified ground sections, at a thickness of approximately 70 μm parallel to the long axis of the implant, were fabricated. The sections were stained with toluidine blue, then observed and pictures were obtained by light microscopy. Using these pictures, the bone contact ratio (BCR) of the implant was calculated. The ratio of the bone observed in the medullary canal was determined as bone density (BD) and also calculated. These histometric procedures were performed using an NIH image and the histometrical data of both the control and experimental groups were statistically analyzed. The unpaired Student's t-test was used to assess the significance.

Findings and Conclusions: in the control group, newly formed bone could be seen surrounding the titanium implants. Newly formed bone was seen to be indirect contact with the implant surface; however, unmineralized connective tissue, including fibroblast-like cells and blood vessels, was occasionally interposed between the bone and the titanium. In the medullary canal, a scanty amount of bone trabeculae could be observed. In the experimental group in contrast, the medullary canal was filled with abundant bone trabeculae with the mesh-like structure. This bone trabeculae seen the medullary canal were somewhat thicker than those of the control group. At the interface, direct contact between the bone and the implant could be also observed; however, despite the fact that the proportion was relatively smaller, connective tissue was interposed between the newly formed bone and the titanium as seen in the control group. Histometrical findings: in the control group, the mean BCR was $31.7 \pm 2.6\%$. in contrast, the mean BCR in the experimental group was $58.4 \pm 5\%$. The mean BD in the control group was $17.4 \pm 4.2\%$; however, the experimental group showed a BD value of $44.3 \pm 6.5\%$. These differences are statistically significant. This report show that simvastatin successfully activated osteogenesis around the titanium implant; however, the information is limited. This drug has been widely used as a cholesterol- lowering drug; however, more information is required for bone regeneration. The optimum dosage, the effectiveness for humans, side effects and other points are to be examined.