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WISSENSCHAFTLICHER ABSCHLUSSBERICHT

Histologische Evaluation des Osseointegrationsverhaltens von oberflächenmodifizierten Dentalimplantaten (UV-C Bestrahlung) aus Zirkoniumdioxidkeramik im Vergleich zu Titanimplantaten am Hausschwein

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Histological evaluation of the Osseointegration of surface modified zirconia and titanium dental implants by UV-C radiation in adult housepigs

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short running title: UVC Implants

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Abstract:

Objective:

The aim of this study was to investigate the effect of ultraviolet radiation type c (UVC radiation) as surface conditioning on titanium (Ti) and zirconia (Zr) dental implants on their bone implant contact.

Material & Methods:

The study was performed in nine adult female domestic pigs over a period of (Group I) 6 weeks, (Group II) 12 weeks and (Group III) 18 weeks. In each animal, eight implants (four Ti and four Zr implants) were inserted into the frontal bone of the pigs. On the right side two Ti and two Zr implants with UVC conditioning (Zr+, Ti+) were inserted and the left side served as control (Zr-, Ti-). Histomorphometric analysis of ground sections and micro-ct were performed and the bone to implant contact was calculated as goal parameter.

Results:

The UVC conditioning made no significant difference ($p>0.1$) in median bone implant contact (BIC) in the first six weeks in both materials (36.4 % Ti+ vs. 40.5 % Ti- and 44.5 % Zr+ vs. 31.4 % Zr-). After 18 weeks, the BIC increased for Ti implants (48.4 % Ti+ vs. 59.5 % Ti-), however, the BIC stagnated and decreased for Zr implants (44.2 % Zr+ vs. 28.5 % Zr-).

Conclusion:

Overall zirconia and titanium implants performed well with no failing. The BIC of zirconia implants profits marginal of the UVC surface conditioning and performed similar to titanium after 18 weeks; however, we could not show a benefit for Ti implants.

Introduction:

Dental implants are widely used for bone-inserted prosthesis. There are lots of different implant systems and different materials, e.g. titanium and zirconia. Dental implants of titanium apply widely to the gold standard in dental implantology because of its biocompatibility including a predictable osseointegration. Improvements in osseointegration are tried by other surface constitutions. A recent purpose is the use of ultraviolet radiation type c (UVC) (peak wavelength of 250 nm) radiation as surface conditioning. In vitro, an improved hydrophilicity and ostesoblastophilicity was showed for titanium and zirconia after UVC conditioning (Aita, Hori et al. 2009; Att, Takeuchi et al. 2009).

Zirconia implants are a possible alternative in cases of patient's aversion to titanium alloys or incompatibility (Sicilia, Cuesta et al. 2008). However, adequate clinical experience and surface conditioning are still missing. Many studies have demonstrated that zirconia implants have a comparable osseointegration and nearly no allergic potential (Andreiotelli, Wenz et al. 2009). Additionally, the plaque accumulation for zirconium dioxide is less than that for titanium (Scarano, Di Carlo et al. 2003). Therefore, zirconia implants with roughened surfaces might be an alternative to titanium implants (Oliva, Oliva et al. 2010; Moller, Terheyden et al. 2012).

The osteoconductivity of titanium alloys and integration in bone is well documented. The bone implant contact is an adequate parameter for histological examinations of osseointegration (Eom, Jeon et al. 2012; Jensen, Schou et al. 2012). Connective tissue enclosing of the implants can also be evaluated histologically as a parameter of quality of integration. The reaction of the bone and tissue, respectively, to the materials of dental implants is important for the success rate of implantation.

The aim of this study was to evaluate the bone implant contact in a time series (6, 12, 18 weeks) after the use of UVC radiation as surface conditioning for titanium and zirconia dental implants in housepigs.

Materials and Methods:

For this study we used nine adult female domestic pigs. Three groups were created with three animals each. The animals of group I were euthanized after six weeks after implants insertion, group II after 12 weeks, and group III 18 weeks.

The dimensions of the used implants were 5 x 10 mm. Titanium and zirconia implants were used (Easy Fast S and Easy Kon, General Implants GmbH, Villingen Schwenningen, Germany). All over, 72 implants (36 titanium and 36 zirconia) were inserted in the frontal bone of the pigs. The surfaces of 18 titanium implants and 18 zirconia implants were conditioned with ultraviolet light type C (UVC) according to the instructions of Aita et al. 2009 before the insertion in the bone (Aita, Hori et al. 2009).

On the right side of the frontal bone the UVC conditioned zirconia and titanium implants (Zr+, Ti+) were inserted and served as test group and on the left side the unconditioned implants were inserted and served as control (Zr-, Ti-). The process of implantation was performed according to the instructions of General Implants (Villingen-Schwenningen, Germany).

After the observation time of 6, 12, and 18 weeks bone blocks were harvested of the frontal bone with the osseointegrated implants and histological examinations were performed. During the observation time the pigs were treated with a protocol of polychrome sequential labelling (calcein [14th day post operationem], alizarinkomplexon

[28th day p. op.], xylenolorange [56th day p. op.], and doxycyclin [77th day p. op.]).

Animal management

Nine adult female domestic pigs (>18 months, average body weight 78.4 kg) were used to perform this study. The animals were kept in small groups in purpose-designed sties and were fed a standard diet (Altromin 9023®, Altromin International GmbH, Lage, Germany) with water provided ad libitum.

The domestic pig is a suitable model for the simulation of operations on humans. The new bone formation rate of the domestic pig (1.2-1.5 µm per day) equals that of humans (1.0-1.5 µm per day) (Honig, Schutt et al. 1999).

Surgical procedure

All surgeries were performed under sterile conditions. For all surgical procedures, the animals were anesthetised with an intravenous injection of ketamine HCl (Ketavets®, Ratiopharm, Ulm, Germany). After applying a local anaesthetic to the operation area (Ultracain D-S forte®, Hoechst GmbH, Frankfurt, Germany), a sagittal incision in the frontal skull was made, and the bone was thereafter exposed via fascia-periosteal flaps. In the lower forehead approximately 8 cm above the level of the eyes sockets for the implants were slowly drilled under copious irrigation at a speed of 500 rpm without pressure using. The implants were inserted 1.0 cm apart to avoid biological interimplant actions (Fig 1).

The implants were inserted in a depth where the implant shoulder was on bone level. After the implant installation, the fascia-periosteal flap and muscle were closed in

separate layers with single resorbable sutures (Vicryl 3.0, Ethicon GmbH & Co KG, Norderstedt, Germany) and the skin was closed with non-resorbable cutaneous sutures (Supramid® 2.0, Resorba. Nürnberg, Germany).

Perioperative antibiosis was achieved with a preoperative intramuscular injection of 1.5 g Augmentan® (GlaxoSmithKline Consumer Healthcare GmbH & Co. KG, Munich, Germany). Postoperative pain control was obtained with one injection of 500 mg metamizol i.m. (Novalgin®, Hoechst AG, Bad Soden, Germany) and oral tramadol 2 x 50 mg/d (Tramal®, Grünenthal GmbH, Aachen, Germany).

Animal euthanasia

The animals were euthanised after a 6, 12, and 18-week observation period. The animals were initially sedated with a mixture of azaperone and midazolam (1 mg/kg, i.m.). Subsequently, a 20 % pentobarbital solution (Dermocal AG, Buenos Aires, Argentina) was delivered into their ear veins until cardiac arrest occurred.

Histology and Histomorphometry

The frontal skull was harvested from each animal, and the specimens were fixed by immersion in 10 % paraformaldehyde solution for 24 hours to render the organic matrix insoluble. Bone segments of 1.5 by 1.5 cm with the dental implant in a central position were cut out using a band saw. All specimens were dehydrated in increasing concentrations of alcohol at room temperature in a dehydration unit (Shandon Citadel 1000, Shandon GmbH, Frankfurt, Germany). The specimens were embedded in Technovit 9100® (Heraeus Kulzer, Kulzer Division, Werheim, Germany) for histological

examination via grounded sections using the technique described by Donath and Breuner (Donath and Breuner 1982).

The histological section was cut out exactly in the longitudinal axis of the dental implant in exactly the frontal plane of the animal. The histological section included the dental implant at the position of its maximum diameter. One central section of each implant was taken. The preparations were prepared as undecalcified hard sections, stained with toluidine blue and examined microradiographically and histologically. One section per implant was fixed on an acrylic carrier and then ground and polished down to approximately 90 microns. Microradiography of the 90 µm specimens was performed on 2 x 2 inch plates (Microchrome Technology Inc., San Jose, CA, USA) at 3 mAs and 25 kV using a microradiography device (Faxitron X-ray systems, Hewlett Packard GmbH, Böblingen, Germany). Using this technique, resolutions of up to one micron can be achieved. At a four-fold magnification, the plates were photographed under the microscope. These slices were composed to provide a total view of the specimens and subjected to qualitative evaluation.

Approximately 40 µm thick sections were stained with toluidine blue for further evaluations, including histometric analysis, and the bone-implant contact was measured. The histological sections were inspected with an Olympus BH2 microscope (Olympus, Center Valley Pennsylvania US) at a magnification of 4x. With a video camera the image was captured and transferred to a histomorphometry workstation (Leica QM500, Solms Germany). Values based on measurements of all threads for each implant were used to calculate mean values for each implant. Starting at the implant shoulder the segment between each thread of the implant was analyzed separately. First the complete implant surface was tracked manually and the length of this line was measured by the system in

mm. Within the analyzed segment parts of the implant surface were then tracked again manually. The length of the intersections containing bone to implant contact was measured by the system. Finally the Bone Implant Contact (BIC) in percent as goal parameter was calculated

Statistical Analysis

The data were statistically analyzed using Winstat for Windows Excel (R. Fitch Software, Bad Krozingen, Germany). The goal parameter was Bone to Implant Contact (BIC) in percentage as described under histomorphometry. Normal distribution of the values was not supposed (Kolmogorov-Smirnov-Test $P<.05$), therefore, data are reported with median (25th-75th percentiles). Statistical testing was performed with a Mann-Whitney-U-Test due to nonparametric distribution. The two hypothese (Zr+ versus Zr-; Ti+ versus Ti-) were tested (significance level of $P\leq.05$) for unconnected samples.

Ethical approval

The investigation was approved by the Animal Ethics Committee at Semmelweis University of Budapest, Hungary (Nr.: 1053/eoh/2007).

Results:

After surgery, all animals exhibited normal eating behaviour. No signs of infection were noted upon clinical examination at any time during the observation period, and the animals gained up to 10% of additional body mass. In addition, no signs of infection were observed in the preparations.

The median values (and 25th and 75th percentiles) of bone implant contact are shown in table 1. The implant success rate stood within the observation period of 16 weeks at 100 percent. No implant failed and all implants of the groups (zirconia implants with and without UVC radiation as surface conditioning / titanium implants with and without UVC radiation as surface conditioning) demonstrated good osseointegration.

The analysis of the micro-ct of all specimens reflected the results of the histology and the histomorphometry (Figure 2). Microradiographically and histologically, no connective tissue sheath was observed on the implant threads. After six weeks, newly formed osteoid and woven bone were evident on the implant threads (Figures 3). This was also evident in the fluorescence microscopy (Figure 4). Close bone-implant contact (BIC) was seen on the titanium, as well as on the zirconium surface circumferential. After 18 weeks of healing, the tissue was re-established as lamellar bone in the contact zone of zirconium and the titanium implants irrespective of modification the surface with UVC radiation (Fig. 5 and Fig 6).

Discussion:

This is the first animal in vivo study testing UVC surface conditioning of titanium and zirconia dental implants. Osseointegration of an osseoconductive dental implant is the

base of implant success rate. An important parameter, beside the primary stability, is the surface roughness (Nasatzky, Gultchin et al. 2003). However, an improvement in surface condition may be an adequate purpose for a higher BIC and implant success rate. Different treatments had been evaluates, e.g. titanium plasma-spraying and acid etching (Novaes, de Souza et al. 2010). The approach of Att et al. (2009) of surface modification with UVC showed a positive influence on the titanium alloy for an improved hydrophilicity and ostesoblastophilicity (Att, Takeuchi et al. 2009). The application of UVC on the implants seems to be a practical chair-side procedure. There are only a few data of in vivo studies regarding surface modification of zirconia implants (Lee, Yeo et al. 2012; Park, Cung et al. 2012; Salem, Taleb et al. 2012). Overall the zirconia and titanium implants performed well with no failing. Our data evaluate a marginal profit of the UVC surface modification of the zirconia implants. There was no benefit in the titanium group. May be a better outcome can be reached through a modified application or combination of different ultraviolet radiations.

However, the interpretation of the current results must consider the study limitations. The small number of animals could be regarded as a limitation of the study. Moreover high interindividual variations of the pigs may also bias the results. Although the number of animals was low, the applied statistical test was able to compensate for the low number and reveals significant differences between the test and the control group.

Conclusion

The BIC of zirconia implants profits marginal of the UVC surface conditioning and performed similar to titanium implants after 18 weeks; however, we could not show a benefit for Ti implants.

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FIGURE LEGENDS:

Figure 1: Experimental design and operation situs in a pig with the positioning of titanium and zirconia implants in the frontal skull.

Table 1: Bone implant contact (BIC) in percentage after the observation period of 6, 12, and 18 weeks. There is no significant difference in BIC between the surface condition with UVC (Ti+, Zr+) and their control (Ti-, Zr-).

Figure 2: Micro-CT with a) mask and b) 3D analysis. The upper 2 mm of the implant were analyzed including the implant threads. Due to the glow effect of the titanium two voxel, directly at the implant, were not evaluated. 1 Voxel corresponds to 10 µm. The analyses included the bone around the implant (cylindric), which corresponds to voxel 3 to 5.

Figure 3: Microradiographic with no connective tissue sheath on the implant threads after a) 6, b) 12 and c) 18 weeks a close bone-implant contact (BIC) was observed.

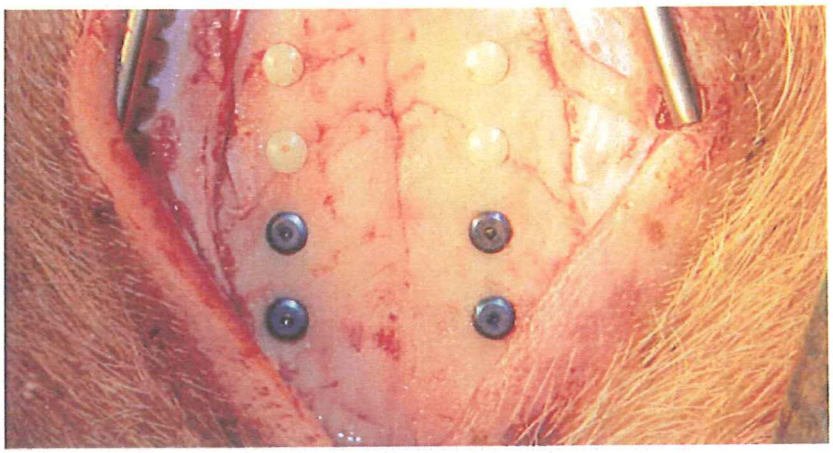
Figure 4: Fluorescence microscopy with signs of osseointegration: already after 2-3 weeks (orange) first bone apposition on the implant surface with interruption of the bands as a sign of bone remodelling.

Figure 5: toluidine blue staining of zirconia implants with surface modification (UVC radiation) = Zr+ and without = Zr- after 6 and 18 weeks observation period. Similar bone to implant contact with circumferential bone formation and osteoid on both surfaces in intimate contact with the zirconia implants (undecalcified hard sections, toluidine blue).

Figure 6: toluidine blue staining of titanium implants with surface modification (UVC radiation) = Ti+ and without = Ti- after 6 and 18 weeks observation period. Same results as with zirconia implants with similar bone to implant contact in intimate contact with the surface (undecalcified hard sections, toluidine blue).

Figures:

Figure 1: Experimental design and operation situs in a pig with the positioning of titanium and zirconia implants in the frontal skull.



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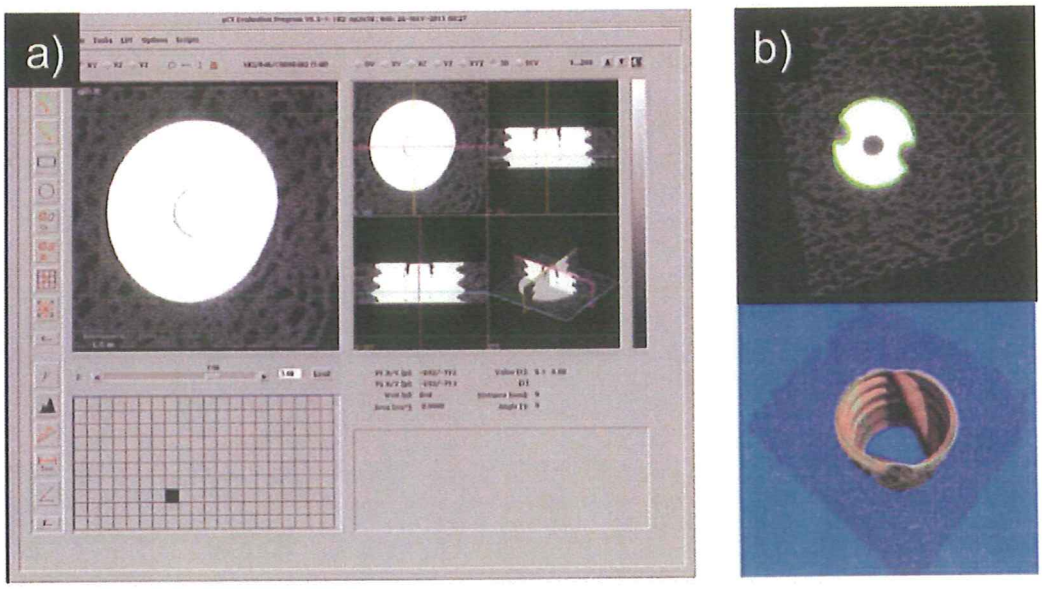
	Bone implant contact			
oberservation period	Ti+	Ti-	Zr+	Zr-
6 weeks	36.4 % (22.0 - 82.5 %)	40.5 % (27.2 - 52.1 %)	44.5 % (25.9 - 63.4 %)	31.4 % (21.9 - 35.3 %)
12 weeks	58.7 % (45.3 - 62.4 %)	51.2 % (43.2 - 65.5 %)	9.9 % (5.4 - 20.5 %)	39.8 % (19.7 - 46.6 %)
18 weeks	48.4 % (29.3 - 62.7 %)	59.5 % (41.0 - 80.3 %)	44.2 % (31.0 - 47.7 %)	28.5 % (/-/)

Figure 2: Micro-CT with a) mask and b) 3D analysis. The upper 2 mm of the implant were analyzed including the implant threads. Due to the glow effect of the titanium two voxel, directly at the implant, were not evaluated. 1 Voxel corresponds to 10 µm. The analyses included the bone around the implant (cylindric), which corresponds to voxel 3 to 5.



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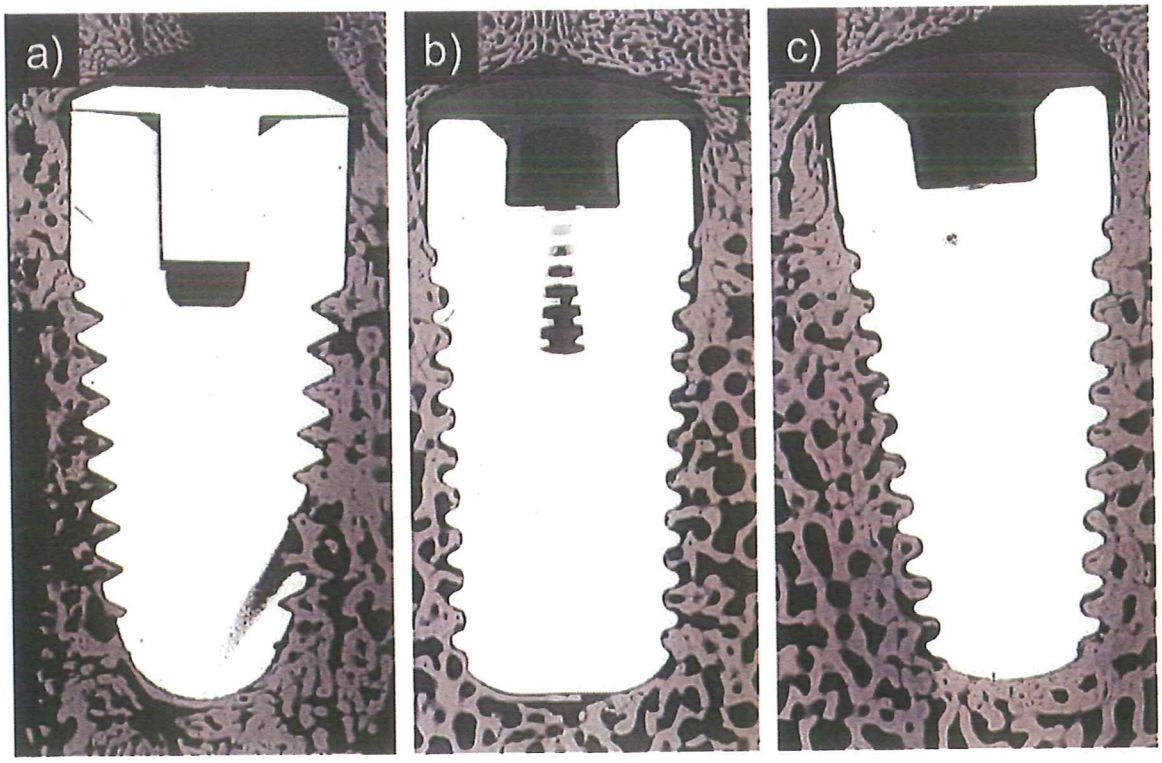

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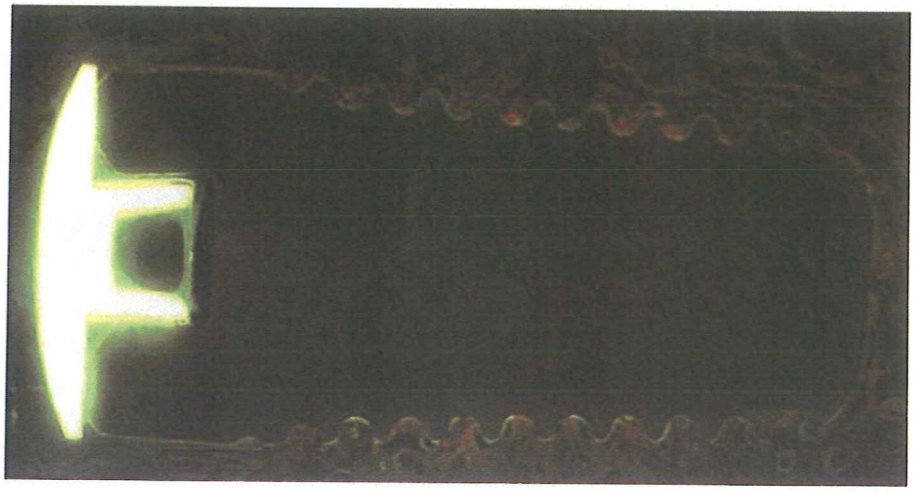

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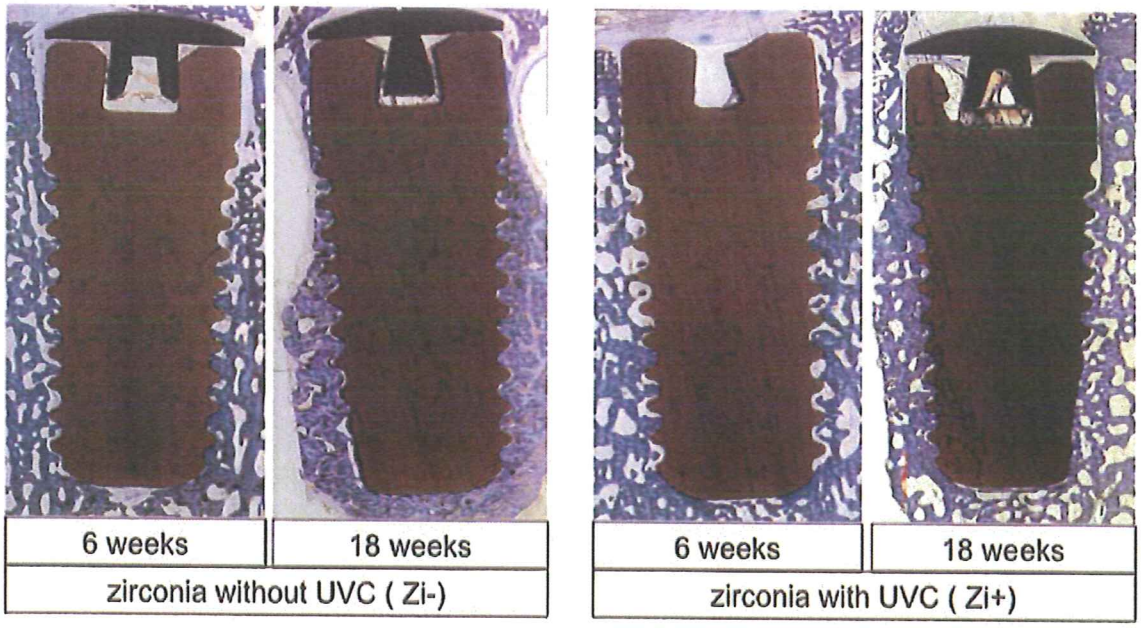

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