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Histomorphometric evaluation of implants coated with enamel or dentine derived fluoride-substituted apatite

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Abstract
Objectives The aim of this study was to compare osseous healing characteristics of titanium implants coated with enamel-derived fluoride-substituted apatite (EFSA) or dentin-derived fluoride-substituted apatite (DFSA).
Methods Fluoride-substituted apatite was derived from extracted human teeth with calcination method at 850 °C. DFSA and EFSA were separated and carefully ground with a blade grinder. Twenty-four titanium implants were prepared from a 99.99% pure titanium bar. EFSA and DFSA powders were sprayed separately on implants. As control group, unsprayed and sandblasted pure titanium implants were used. Eight adult rams were used in the study. One EFSA coated, 1 DFSA coated and 1 control implants were placed into right tibia of each rams. The rams were sacrificed after 6 months of healing. Undecalcified sections were prepared according to Donath's method and histomorphometric evaluation of implants was made.
Results The mean bone contact percentage of DFSA-coated, EFSA-coated and control implants was 89.88% ± 2.34, 70.19% ± 13.11 and 53.12% ± 5.76 respectively. This study suggests that DFSA-coated implants achieved better bone contact than EFSA-coated implants ($P < 0.05$). Also study groups presented better bone contact than control group ($P < 0.05$).
Conclusions The results of this study show that although DFSA-coated implants achieved better bone contact, both DFSA and EFSA can be considered as appropriate coating materials.

Introduction

The utilization of dental implants for the rehabilitation of completely or partially edentulous patients has become a standard treatment modality in dentistry. Branemark (1983) defined the contact interface between organized living bone tissue and the surface of the implant as a functional and structural unit (osseointegration), based on the observation of direct bone apposition to screw-type machined implants of commercially pure titanium [1]. Surface topography plays a critical role in the interaction of dental implants with adjacent tissues [2–5]. The implant surfaces and types most frequently described in the literature may be subdivided into implants with roughened surfaces with a coating [e.g. titanium plasma-sprayed (TPS) or hydroxyapatite coated (HA)], implants with machine-processed (e.g. machined or polished) titanium surfaces without a coating, and implants with microroughened surfaces without a coating (e.g. sand-blasted or acidetched). In recent years,

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there has been a tendency to replace titanium plasma-sprayed surfaces by sandblasted and acid etched surfaces in order to accelerate osseointegration [3, 4, 6–9].

In addition, the osseous healing characteristics of various surface structures have been investigated in several experimental and clinical studies [10–14]. In vitro studies have shown that microroughened sandblasted and/or acidetched surfaces enhance platelet activation and aggregation and fibrin retention [3, 4, 15]. Animal studies have demonstrated an enhanced bone implant contact and removal torque values with microroughened surfaces compared to smooth and TPS surfaces [8, 9, 16–18]. Although, improved success rates have been reported in low-quality bone such as posterior maxilla [19, 20], clinical studies have demonstrated that posterior maxilla have a significantly lower success rate compared to areas of denser bone [8, 21, 22]. Therefore, another approach to improve osseous integration of dental implants in these anatomical regions has been the utilization of calcium phosphate coated implants since these coatings have been found to accelerate initial stabilization of implants by enhancing bony ingrowth and stimulating osseous apposition to the implant surface [4, 7, 23–26]. Furthermore, recent clinical trials have shown higher long-term success rates for HA-coated implants compared to machined titanium implants as well as an accelerated initial rate of osseointegration [27, 28].

Living in the era of life control and prolongation, artificial implants of hydroxyapatite (HA), with the chemical formula, $Ca_{10}(PO_4)_6(OH)_2$, are very popular for hard tissue (e.g., bone) restorations because they accelerate bone growth around the implants [23–26]. Biological apatites attract special interest since it is believed that the several substitutions at the Ca^{2+}, PO_4^{3-} and OH^- sites of HA and the presence of several trace elements play an important role in the overall physiological functioning and in the osseointegration process [23–26]. The poor mechanical properties of pure biomaterials, such as HA, have directed biomaterials design to tissue-engineering approaches [29]. HA has been widely investigated and most commonly used for coating dental implants [23–26]. HA is one of the most widely used biomaterials for reconstruction of the skeleton. HA is nontoxic and biocompatible with bony tissues. It is used as an implant material both in its bulk form and as a thin coating on metals. HA ceramic surfaces can achieve direct bonding with bone [24].

HA can also be hydro-thermally converted from natural sources such as coral and human/animal mineralized tissues or synthetically manufactured [30]. The physical properties of HA make it favourable for osseointegration with host bone [31]. Although the main chemical formulation is similar, natural HA crystals are relatively small and orientation of its crystals might differ as compared to synthetic one [30–33]. It is already known that the crystal structure can alter the dissolution rate and influence the cell behaviour in the presence of the biomaterial [30]. Such natural apatites as bone and teeth contain trace elements like fluoride. The mineral phase of tooth enamel and dentine consist of apatite containing high level of fluoride. It is also correct to describe tooth derived HA as fluoride-substituted apatite.

The physical and chemical properties of enamel fluoride-substituted apatite (EFSA) and dentin fluoride-substituted apatite (DFSA) were reported in a recent study [30]. Unfortunately there is a lack of evidence based studies on osseous healing characteristics of EFSA and DFSA.

The aim of this study was to compare osseous healing characteristics of titanium implants coated with EFSA or DFSA.

Material and methods

Production of HA and implants

The HA material used in this study was derived from freshly extracted human teeth. Due to high amount of Hg has been found in a previous study [30], teeth with fillings and caries were excluded. The teeth were irrigated with tap water and soaked in 1 % concentration of antiseptic solution (15% Cetrimide and 1.5% Chlorhexidine) for 10 minutes to prevent bad odour and contamination of various infectious diseases. After the reirrigation process, teeth were deproteinized in an alkali solution (1% concentration of sodium hypochloride). Samples were reirrigated with tap water again. All the samples were calcined at 5 °C min^{-1} to 850 °C and kept for 5–6 h. It was observed that at high temperatures, the dentin and enamel materials were separated easily as 60% dentin and 40% enamel. The enamel and dentin particles were ground with a blade grinder for 30 s. Samples were poured into sieves placed from coarsest to finest in series and the particles between 106 and 150 μm in size were used in the study. The details of the method used to extract the enamel and dentin powders were described elsewhere [30].

Twenty-four titanium implants, 6 mm in length and 6.35 mm in diameter were prepared from a 99.99% pure titanium bar. All implants were mounted on a round steel rode for continuous plasma spraying process. EFSA and DFSA powders were sprayed separately on titanium implants. As control group, unsprayed and sandblasted pure titanium implants were used. All implants were sterilized in an autoclave.

Animals and surgical procedure

In this study, eight skeletally mature, mixed-bred rams aged 2 years old, weighing an average of 51.9 kg

(48.9–55.6 kg) were used. The study was approved by the local Animal Ethics Committee. All the animals were used in the study according to the Guidelines of Marmara University Care and Use of Laboratory Animals. Anesthesia was induced with intravenous injection of 1.0 g pentobarbital sodium (Pental Sodyum®, İbrahim Etem, Turkey) and maintained with a halothane (2–3%) (Halothan®, Aventis Pharma, Turkey) and oxygen (2 L/min) mixture. Prophylactic and postoperative antibiotics (Klindan® 600 mg, Bilim, Turkey) were administered for 3 days.

Implantation was carried out under standard aseptic surgical conditions. Marcaine 0.5% (Bupivacaine®, Eczacıbaşı, Turkey) was injected (2.0 mL) before starting the surgical incision medially over the distal femur and the proximal tibia. After reflection of surgical flap, three holes were drilled under copious saline irrigation. The holes were prepared in the proximal tibia in 7 mm depth using a drill set (2.0, 3.0, 3.8, 4.5, 5.5, 6.5 mm in diameter). The designated implants were then inserted in press-fit using finger pressure. One EFSA coated, 1 DFSA coated and 1 control implants were inserted into the right proximal tibia of each rams. A cover screw was inserted into each implant and the mucoperiosteal flaps were sutured primarily to ensure complete wound closure. Wounds were closed in layers and Marcaine 0.5% (Bupivacaine® Eczacıbaşı, Turkey) was applied to each site. A long acting opioid (buprenorphine HCl, 0.015 mg/kg i.m b.i.d for 3 days) was administered for pain control. All the animals were sacrificed 6 months after implant placement with an overdose of pentobarbital sodium injection (100 mg/kg i.v).

Specimen preparation

The right tibiae were removed. After being dissected from the adhering soft tissues, the proximal one third and a part of the distal tibiofibular junction of the tibiae were cut. The specimens were trimmed under isotonic saline irrigation within 4 mm of the implant surfaces subsequently all the specimens were fixed in 10% neutral buffered formalin for 48 hours and dehydrated in ascending concentration of ethanol 70–99.9% under vacuum. The dehydrated specimens were embedded in methylmethacrylate (Tecnovit® 7200 VLC. Heraeus Kulzer GmbH & Co. KG, Wehrheim, Germany) without decalcification. The resulting blocks of the specimens were sectioned longitudinally from the centre of the each implant. Approximately 200 μm thick parallel sections were cut from the blocks by using a band saw (Exakt 300 CL, Exakt Apparatbau, Norderstad, Germany). The 200 μm thick sections, which were mounted on the slides, attached to the micro parallel grinding system (Exakt 400 CS, Exakt Apparatbau, Norderstad, Germany). The 50 μm thick final slides were prepared by grinding the sections with descending 320–4,000 grit sandpapers (Hermes Schleifmittel GmbH & Co. KG, Hamburg, Germany). Four slides were prepared for each implant. Sections of all implants were obtained sequentially through the same plane. Then each slide was stained using toluidine blue.

Bone histomorphometry

The images were captured via light microscope at 4× enlargement attached a digital camera system (Olympus BX50, Olympus Optical Co. Ltd. Tokyo, Japan). The images then transferred to the PC (Pro2000, Turkey). All measurements were made using the Image J v1.29 (W, Rasband, NIH, USA) image analysis software. Bone histomorphometry concept and technique have been described elsewhere [34]. The length of direct contact between the bone and the implant was measured over the entire implant perimeter. The measured value was expressed as a percentage of the axial perimeter. The resulting measurement is referred to as the percent bone contact length. Differences in the percent bone contact length between implants from the different study groups were statistically compared. SPSS 14.0 package program was used for statistical analysis, and the repeated measurement analysis, which is a member of General Linear Model was applied. Statistical significance level was determined by 0.05.

Results

Uneventful healing was achieved in all of the animals. No implants were lost during the healing phase. The results from the histomorphometric analysis are presented in Fig. 1. The mean bone contact percentage of DFSA-coated implants was 89.88% ± 2.34, and the mean bone contact percentage of EFSA-coated implants was 70.19% ± 13.11. Direct bone-implant contact was found at 53.12% ± 5.76 for the control group. The implants in study groups exhibited a high level of osseointegration (Fig. 2 and 3). The study groups presented statistically significant better bone contact than the control group ($P < 0.05$) (Fig. 4). Also, DFSA coated implants achieved a statistically significant higher level of osseointegration as compared to EFSA-coated implants ($P < 0.05$).

Discussion

In implant dentistry, there has been an ongoing effort to enhance and accelerate osseointegration of dental implants by coating their surface with different materials. This is related to the fact that treatment outcomes in dental implantology critically depend on implant surface design

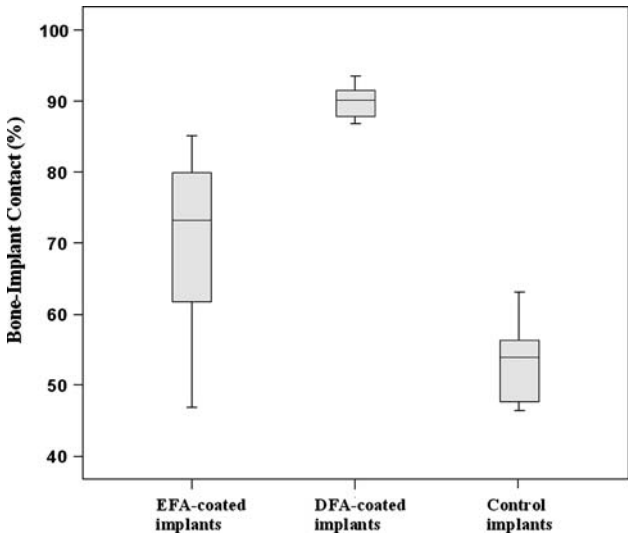

Fig. 1 Box-plot graphic shows the bone-implant contact percentages of EFSA coated, DFSA coated and control implants

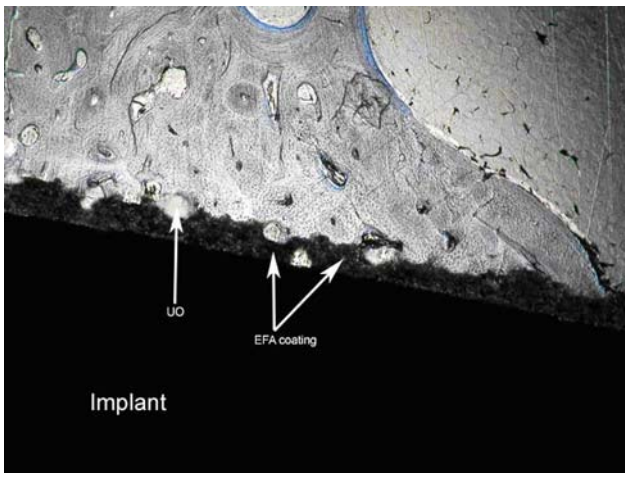

Fig. 3 Undecalcified ground sections of the EFSA coated implant which was examined after 6 months of healing. Although a high osseointegration rate can be demonstrated, there are several unmineralized osteoids (UO) between the bone and the implant (Toluidine blue, original magnification ×4)

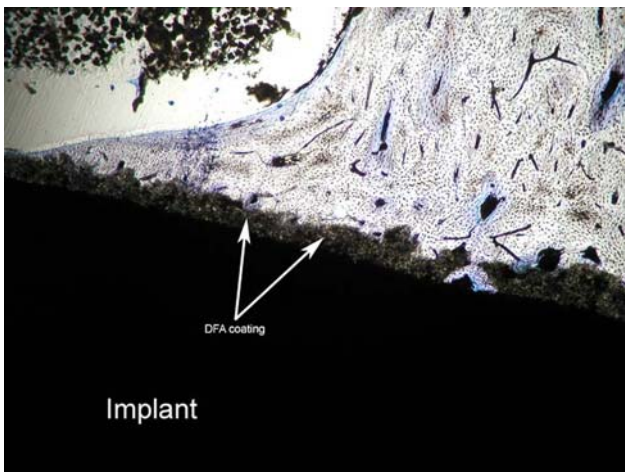

Fig. 2 Undecalcified ground sections of the DFSA coated implant which was examined after 6 months of healing. The well organized interface between the bone and DFSA coating implant can be seen. There is no unmineralized osteoid (UO) between the bone and the implant. (Toluidine blue, original magnification ×4)

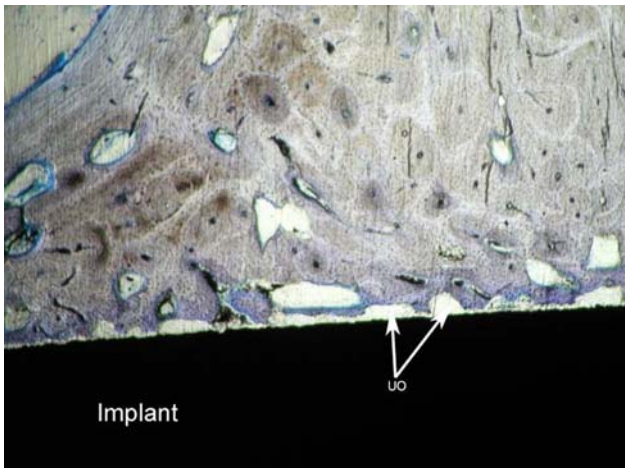

Fig. 4 Undecalcified ground sections of the sandblasted pure titanium control implant which was examined after 6 months of healing. Although there is no fibrous tissue present in the interface, bone-implant contact was found remarkably low than the study groups. (Toluidine blue, original magnification ×4)

[4, 35]. The influence of implant surface on osteoblastic cell differentiation is an important aspect. To enhance osseous integration, dental implant surfaces should have the ability to stimulate differentiation of osteogenic cells and matrix formation at their surface. Hydroxyapatite (HA) deposited on the surface of titanium alloy by plasma spraying has been widely used in orthopedics and dentistry because of its excellent biocompatibility. HA coating was able to bond directly to bone without an intervening fibrous layer at interface due to the chemical resemblance of HA to bone minerals [36] The excellent bone bonding and osteoconductive capacities of HA have been extensively described elsewhere [37, 38]. Also high level of osseointegration has been reported by HA coated implants [23–26, 39, 40].

However, there has been no consensus on the long-term performance of HA coatings on dental implants. High temperatures (1,550 °C) held in plasma spraying, usually far beyond HA melting point, inevitably causes decomposition of HA and changes of crystalline phases [11]. Furthermore, rapid cooling from molten state to room temperature causes formation of amorphous calcium phosphate on coating. Therefore, the resulting HA layer after plasma–spray may be subjected to biodegradation and

bioresorption over prolonged implantation periods, probably accelerated by micromotions of the implant, resulting in loosening of mechanical fixation and delamination of the coating [12]. These phenomena certainly affect the long-term stability of plasma-sprayed HA coatings [13].

It has been hypothesized that a change in the chemical composition of HA by replacing the hydroxyl-group with fluoride ions, may improve the stability of apatite coatings after plasma spraying process. Fluoroapatite is known with its high thermo stability and low solubility [41]. In vivo studies demonstrated that fluoroapatite coatings are stable and do not show any signs of dissolution or degradation [42–44]. Also other studies on fluoroapatite, and HA demonstrated an equal degree of bone apposition and less coating dissolution within the first 3 months of implantation [43–45]. Caulier et al. found no significant differences in bone reaction between fluoroapatite and HA coated implants which were placed in the maxilla of goats [46]. In addition, low coating solubility and degradation rate for fluoroapatite were stated in other studies [47, 48]. Furthermore, the use of fluoride in HA produces more gradual coating resorption with the added benefit of stimulating bone growth leading to improved osseointegration. Fabrication of mechanical assemblies of various ratios of fluoroapatite and HA has shown potential to include the beneficial effects while enhancing the mechanical properties of the manufactured body [48]. Thus, fluoroapatite coatings could be a promising alternative to HA coatings.

Material aspect of characterisation of the EFSA and DFSA has been extensively studied by scanning electron microscope (SEM), energy dispersive x-ray spectroscopy (EDXS), wet chemical, ion chromatography (ICP), atomic absorption, x-ray diffraction and infra red (IR) methods [30]. Oktar etal. examined the EFSA and DFSA samples by SEM. and reported that dentin particles were much coarser than enamel. The samples were compared with a synthetic HA sample. It was observed that all the samples meet the American Society for Testing Materials (ASTM) designation of F 1185–88 specification [30]. In addition, EDXS analysis of EFSA and DFSA revealed that elemental compositions are Mg 0.04%, Si 0.14%, P 15.19% and Ca 44.73%. and Mg 1.1%, Si 0.22%, P 13.18%, Ca 47%, respectively. Since the EDXS is regarded as a semi qualitative analysis method, the samples were subjected to wet chemical analysis [30]. In order to determine the exact F amount, ICP method was employed. The ICP results were for EFSA and DFSA samples 206.795 ppm and 211.480 ppm respectively [30]. Atomic absorption analysis was applied to heavy metals (Pb, Hg, Cd) regarding the ASTM designation of F 1185–88 specification in a previous study [30]. Also amalgam alloy residues like Ag, Sn, Cu, and Zn were checked in the same study. Although relatively high amount of Hg (EFSA:39.97 ppm, DFSA:21.29 ppm) was found, the total amount of heavy metals was within the limit [30]. This high amount of Hg can be attributed to amalgam fillings. Amalgam residues may have been left in the dentin and enamel particles. X-ray diffraction analysis was based on ‘‘Joint Committee on Powder Diffraction Standard’’ and most strong peaks belong to HA. Furthermore, some unimportant small alpha or beta-tricalcium phosphate (TCP) peaks were observed [30]. It is clear that the calcinations of bone apatite to 800 °C yields formation of beta-TCP [34]. The results of IR absorption analysis of EFSA and DFSA were similar. In addition, EFSA and DFSA were not different from synthetic ones [30]. The biocompability and mechanical properties of the EFSA and DFSA have also been also reported elsewhere [35, 36]. DFSA and EFSA are load-carrying biomaterials according to their physical properties [35, 49]. EFSA has higher compressive strength values than sintered DFSA [49]. Table 1 summarizes the tensile test results of various materials.

Although synthetic fluorapatite has been considered as a successful coating material in implant dentistry, there is a lack of evidence based studies using natural fluoride-substituted apatite as an implant coating material in the literature. In this present study, the osseous healing characteristics of EFSA and DFSA produced from natural sources were evaluated as a coating material on implant surface. The results show that a high level of osseointegration can be achieved by EFSA and DFSA as 70.19% ± 13.11 and 89.88% ± 2.34 respectively following a 6 months osseointegration interval. The performance of HA-coated implants regarding bone-implant contact percentage is consistent with the previous studies [23–26, 39, 40].

Table 1 Tensile test results of various materials

Material type		Tensile test results	
	Particul size range	Simple	Bond
Human derived HA (HHA) [51]	60–99	6.91 ± 1.65	13.20 ± 1.70
Bovine derived HA (BHA) [50]	150–200	9.34 ± 1.45	10.20 ± 2.13
Dentine derived FA (DFA)[49]	75–250	6.27 ± 1.25	7.84 ± 1.60
Enamel derived FA (EFA)[49]	75–250	6.66 ± 1.82	9.71 ± 1.43
AMDRY 6021[49]	45–160	11.49 ± 0.21	12.05 ± 0.52

Conclusion

Within the limitations of this study, EFSA and DFSA can be used as a coating material on dental implant surfaces. Due to simple, low cost production method and high level of osseointegration, capability EFSA and DFSA can be alternative coating materials. The trace amount of F content and modification of these materials with Ti, Zi, or bioglass composites could be leading factor for further studies.

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