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Effects of local application of the ankaferd blood stopper on osseointegration in three different surface titanium implants

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ABSTRACT

Objective: Researchs of the effects of ankaferd blood stopper (ABS) on bone healing metabolism have revealed that it affects bone regeneration positively. The exact mechanism by which this positive effect on bone tissue metabolism is not known. The aim of this study is to biomechanic and biochemical analysis of the effects of the local ABS application on osseointegration of 3 different surfaced titanium implants.
Material & Methods: Spraque dawley rats were divided machined surfaced (MS) ($n = 10$), sandblasted and large acid grid (SLA) ($n = 10$) and resorbable blast material (RBM) ($n = 10$) surfaced implants. ABS applied locally during the surgical application of the titanium implant before insertion in bone sockets. After 4 weeks experimental period the rats sacrificed and implants with surrounding bone tissues were removed to reverse torque analysis (Newton), blood samples collected to biochemical analysis (ALP, calcium, P)
Results: Biomechanic bone implant contact ratio detected higher in SLA surfaced implants compared with the RBM and controls ($P < 0,05$). Phosphor levels detected lower in RBM implant group compared with the controls and SLA ($P < 0,05$). Additionally; phosphor levels detected highly in controls compared with the RBM implants.
Conclusion: According the biomechanical parameters ABS may be more effective in SLA and RBM surfaced implants when locally applied.

1. Introduction

In the treatment of partially and completely edentulous patients, implants made to replace missing teeth are used routinely. This treatment procedure was developed in the late 1960s and early 1970s by Brånemark et al. and Schroeder et al.,[1–3] who later studied the regular structural and functional connection between living bone tissue and the implant surface now known as osseointegration. Since then, research has concentrated particularly on two important issues, namely how to improve the osseointegration process and how to increase the long-term integration of dental implants into bone.[4]

The literature reports many implant products that increase the surface porosity of materials by various means to enhance osseointegration. Not all of these methods yield a positive effect on repair, so more detailed research is needed, not only from a mechanical or pharmacological perspective, but also in terms of viable tissue response and biocompatibility, in order to find the most suitable method for guaranteeing osseointegration and regeneration, ensuring the mechanical integrity of the connection between implant and bone.[5–11]

Ankaferd Blood Stopper (ABS; Ankaferd Medication Cosmetics AS, Istanbul, Turkey), is a plant extract used an antihemorrhagic agent, obtained from *Glycyrrhiza glabra, Vitis vinifera*, *Alpinia officinarum*, *Urtica dioica*, and *Thymus vulgaris.*[12]

ABS creates a protein network by clustering protein molecules, especially fibrinogen, in areas where bleeding occurs. Erythrocytes, thrombocytes, and red blood cells are involved in the formation of the

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protein network. Studies have reported that there is no negative effect on any coagulation factor while the hemostatic plug is formed, and it occurs as a completely physiological process.[13] ABS reportedly assists bone healing and has anti-inflammatory and antioxidant effects.[14,15]

The success of dental implants depends on the quality of bone–implant fusion. The surface properties of dental implants are among the important parameters affecting such fusion. Recent implantology studies have focused on the improvement of implant surface properties. Implant surfaces can be classified into six groups: titanium plasma spray-coated surfaces; sandblasted and acidified surfaces; sandblasted surfaces; sandblasted, large-grit, acid-etched surfaces (SLA); hydroxyapatite-coated surfaces; electro-polished (oxidized) surfaces; and surfaces prepared by machine.[16]

The literature contains many studies about coating implant surfaces with an antibacterial layer. Antibiotics such as gentamicin, vancomycin, tobramycin, and silver are the main materials used for this purpose. Silver is an element with well-known antimicrobial activity and is used in medicine in materials such as vascular, urinary, and peritoneal catheters, vascular grafts, heart valve prostheses, and suture materials.[17]

The aim of our study is to biomechanically and biochemically evaluate whether topical ABS application has any effect on osseointegration during the placement of dental implants with different surface structures.

2. Materials and methods

The experiments in our study were carried out at the Firat University Experimental Research Center (Elazig, Turkiye). Ethics committee approval of the study was given by the Firat University Animal Experiments Local Ethics Committee (16.06.2020-396483). The experimental animals (rats) used during the experiment were taken from Firat University Experimental Research Center. The procedures in the experiment fully complied with the principles of the Helsinki Declaration. Thirty Sprague Dawley rats were used in the study, divided into three groups of 10. The rats were housed in places with 55% humidity and a controlled temperature of 22 ± 2 °C, on a 12 h light/12 h dark cycle. The rats were kept in standard cages in threes and fed ad libitum with normal diet and water.

2.1. Surgical procedure

The rats were not fed for 8 h before the dental implant application. The surgical procedures were performed under general anesthesia and using sterile instruments. Xylazine hydrochloride (Rompun®, Bayer, Germany) and ketamine hydrochloride (Ketasol®, Richter Pharma, Austria) were chosen as general anesthetics. Local bleeding control was achieved using mepivacaine hydrochloride (0.3 ml/kg, 2% with scandicaine epinephrine 1: 100.000, Septodont, France). In order to reach the area where the dental implant was to be applied, the fur in the application area was cleaned and then the area washed with povidone iodine. After making a 1.5 cm incision over the tibial crest, the area where the dental implant was to be applied was reached with the help of a periosteal elevator (Fig. 1). The implant socket was opened to the corticocancellous bone in the metaphyseal parts of the right tibia bones of the subjects with the help of burs. The sockets were filled with ABS (Turkish Patent No. 2007-0-114485) agent before titanium implants (Implance Dental Implant Systems, AGS Medical, Istanbul, Turkiye) (diameter: 2.5 mm, length: 4 mm) were placed in them (Fig. 2). After the placement of the implants, absorbable suture (5/0 vicryl, Ethicon, Inc., USA) was used to cover the soft tissues and monofilament suture (Nylon 4.0, Ethicon, Inc.) to cover the skin. 45 mg/kg penicillin as an antibiotic and 0.2 mg/kg tramadol hydrochloride as an analgesic agent were administered intramuscularly at a single dose per day for 3 days for infection and pain control. The rats were sacrificed after 4 weeks. While blood taken from rats after sacrification was evaluated biochemically, implant osseointegration was evaluated biomechanically.

Machined surface (MS) implant group (control group, n = 10): Titanium implants ($TiAl_6Va_4$) implants, 2.5 mm in diameter and 4 mm in length, with a machined surface were surgically placed in the metaphyseal parts of the right tibia bones of the subjects. Immediately before insertion, ABS was applied to the implant sockets to the maximum quantity possible and implants placed immediately after. No additional treatment was applied during the 4-week experimental setup.

Fig. 1. Surgical applications. The approach of the metaphyseal part of the right tibial bone after incision and dissection of the soft tissues and periosteum.

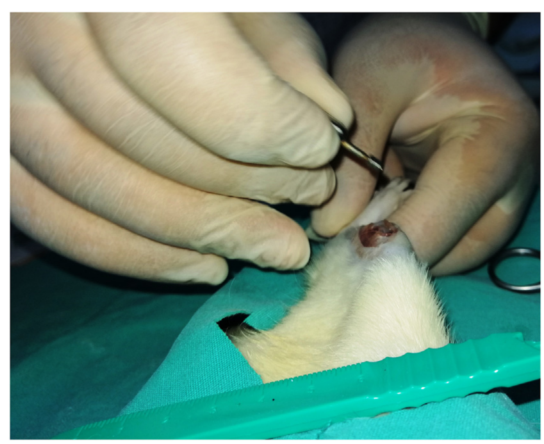

Fig. 2. After the surgical creation bone cavities and integration of the titanium implants.

Sandblasted acid-etched surface implant group (SLA group, n = 10): $TiAl_6Va_4$ implants with a 2.5 mm-diameter and a 4 mm-long sandblasted acid-etched surface were surgically placed in the metaphyseal parts of the right tibia bones of the subjects. Immediately before insertion, ABS was applied to the implant sockets to the maximum quantity possible and implants were placed immediately after. No additional treatment was applied during the 4-week experimental setup.

Resorbable Blast Material Surface implant (RBM) group, n = 10): $TiAl_6Va_4$ implants with a 2.5 mm-diameter and 4 mm-long surface roughened with fusible material were surgically placed in the metaphyseal parts of the right tibia bones of the subjects. Immediately before insertion, ABS was applied to the implant sockets to the maximum quantity possible and implants were placed immediately after. No additional treatment was applied during the 4-week experimental setup.

2.2. Biomechanical analysis

The rats were sacrificed 4 weeks after the surgical procedure, and biomechanical tests were started without delay. The reverse torque method was used in the biomechanical tests. The block bone containing the implants placed in the tibia was obtained for the performance of the tests. Samples were kept in a liquid solution of 10% buffered formalin until the test setup was prepared. In order to prevent the dehydration that may occur in the bone tissue, the evaluation process was started immediately. All of the implants placed in the tibia were embedded in polymethylmethacrylate blocks. An application apparatus was employed in order to measure the reverse torque of the implants. Then, using a digital torque tool (Mark 10, NY, USA), a counterclockwise extraction force was manually applied slowly and increasingly (Fig. 3). When the rotation of the dental implant in the bone socket in the tibia ceased, the torque procedure was stopped immediately. At the time of the break, the highest torque force (Ncm) obtained by the digital torque device was automatically recorded.

Fig. 3. Biomechanic analysis (Reverse torque) of the samples (Mark 10, NY, USA).

2.3. Biochemical analysis

Blood samples were obtained from the rats before sacrification by cardiac puncture without anticoagulant. Later, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, calcium (Ca), phosphorus (P), serum alkaline phosphatase (ALP), and calcium (Ca) values were measured individually. Biochemical analyses were performed in the central biochemistry laboratory of Firat University Faculty of Medicine.

2.4. Statistical analysis

Statistical analysis was performed with SPSS 20.0 Windows software (IBM, USA). Whether the data were normally distributed was determined using the Kolmogorov - Smirnov test. One-way anova was used in groups with normal distribution. Tukey's honestly significant difference (HSD) test was used to determine the group that caused the differences, and $p < 0.05$ was considered statistically significant in the analyzes.

3. Results

As shown in supplemental Table 1; the biomechanical analysis revealed that the mean value was 1.42 ± 0.77 in the machined surfaced implants, 2.32 ± 0.66 in the implants with SLA surface, and 1.11 ± 0 in RBM surfaces. As shown in supplemental Table 1; in the statistical analysis, a statistically significant difference was found between the controls and the SLA group ($P < 0{,}05$) and between the SLA group and the RBM group ($P < 0{,}05$). In addition, as shown in supplemental Table 1; the biochemical analysis showed significant differences between the groups in P. As shown in supplemental Table 1; in SLA group phosphor values detected higher compared with the controls and RBM ($P < 0{,}05$), and in RBM phosphor values detected lower compared with the control group $P < 0{,}05$). As shown in supplemental Table 1; statistically significant differences were not detected between the groups in Ca and ALP values ($P > 0{,}05$).

4. Discussion

Many surface modification methods are employed to increase the osseointegration of dental implants. Such methods can include abrasion of the outer layer, bioactive coating applications, chemical applications.

The simplest and most common method used is known as sandblasting.[18,19] This method contributes to osseointegration by increasing the osteoblast bonding speed between the dental implant and the bone tissue. In this method, there must be no sand particles on the implant surface after sandblasting, as they may cause inflammation in the implanted tissue.[20,21]

In the sandblasting methods, negative load formation occurs on the titanium implant, increasing the osseointegration between bone tissue and implant tissue.[22] Guo et al.[22] suggested that the titanium plates applied to dissimilar groups were sandblasted with aluminum oxide (Al2O3) and calculated the static voltage values and showed the entity of negative static voltage. Modification even a minor parameter may result in very dissimilar surfaces and structures, which should not be ignored in the formation and improving of the surface.[23,24] The sandblasting method changes not only the mechanical but also the chemical structure of the dental implant surface, as well as increasing the wettability and potential of the titanium implant surface for interaction with biologic fluids.[25,26] The most used material used in modifying the surface of dental implants is Al_2O_3. It has been suggested that, in this method, osteoblast behavior changes in bone attachment and organized roughness values are acquired with particles of different sizes.[27,28] Bushinsky et al. suggested that Al_2O_3 stimulates Ca flow through the bone.[29]

Dental implants contain materials such as hydroxyapatite powders, titanium dioxide (TiO_2), silicate glass, which are used as an alternative to Al_2O_3 in the surface modification method. In a dog implanted study, it was reported that the TiO_2 sandblasted surfaces had more anchorage than a machined surface, but there was no difference in the bone–implant contact value.[30]

Choi et al. evaluated bioactive glass particles, silicon dioxide (SiO_2) sandblasted titanium plates, and non-sanded plates. They found that the highest roughness rate occurred in SiO_2 sputtered plates.[31] A rough titanium surface promotes protein adhesion and the differentiation of bone cells.[32,33]

Yurttutan et al. evaluated bone implant osteointegration at 1 month and 3 months in Al_2O_3, TiO_2, SiO_2 sandblasted implants and unpolished treated surface implants (control). They reported that while there was no statistically significant difference between the groups in primary stabilization values, the average implant stability ratio (ISQ) was higher in implants sandblasted with Al_2O_3.[30]

In recent studies, bone healing time has reportedly been shortened by using various modified surface treatment techniques, including sandblasting, acid etching, anodizing, plasma spraying, and coating with inorganic calcium phosphate, drugs, or biological molecules. Chemical modifications may shorten the time required for implant loading, as they enhance biological properties that promote osseointegration and bone formation. In a study that was evaluated histologically after 12 weeks of follow-up, the researchers reported that increased BIC and neovascularization occurred around the implants in the statin group.[34]

Implant surfaces are designed to increase the binding of target tissue cells and prevent bacterial adhesion. The main strategy for reducing bacterial infiltration on the implant surface is to increase the antibacterial ability of the surface and to reduce its bacterial compatibility.[35] In the study of Zhao et al. in vitro and in vivo studies were conducted to evaluate antibacterial coatings on titanium-based implants. Although they concluded that progress was being made in such antibacterial coatings, there is no evidence yet that they have featured in a wide range of clinical uses. In vivo information on antibacterial coatings is still scarce.[36]

Göker et al. evaluated the effects of ABS on bone healing in defects created in diabetic animals, and reported that ABS creates an encapsulated protein network that provides binding sites, which assist in the collection of red blood cells.[12] Bulut et al. suggested in their study that ABS increased bone healing in both diabetic and non-diabetic rats. In addition, they reported that using ABS locally in bone defects did not cause a foreign body reaction. Inflammation and necrosis decreased and new bone formation increased in early bone healing. They emphasized that ABS can be used safely in oral surgical procedures in patients with wound healing disorders.[37]

5. Conclusion

In our study, we evaluated the effect of ABS application on the surface structure of implant types with different surface structures. Our analyses showed that, when ABS was applied in the control group and in the SLA group in bone–implant connection, more osseointegration occurred with the SLA surface. We think that this was due to the increase in the surface area of the SLA surfaces owing to acidification, consequently increasing ABS retention and preserving their antioxidant, anti-inflammatory, and antibacterial properties. In addition, in the comparisons between the SLA and RBM surfaces, the osseointegration value of the SLA surface was found to be higher, thanks to less ABS involvement because of the coating of the RBM surfaces. Also, there was no significant difference between the control and the RBM surface groups. We think that the SLA implant surface may be more successful in ABS applications. Further studies are needed to clarify the association of implant surface-ankaferd application and osseointegration.

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Declaration of competing interest

The authors declare there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jobcr.2021.07.008.

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