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Effects of local enemal matrix protein on osseointegration of different surface Titanium implants

Efectos de la proteína de la matriz de esmalte local, sobre la oseointegración de diferentes implantes de Titanio de superficie

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ABSTRACT

The aim of this study is to investigate the osseointegration levels of implants with different surfaces locally applied with enamel matrix protein by biomechanical methods. Thirty adult female Spraque Dawley rats weighing 300–350 g were included in the study as subjects. The rats were divided into 3 groups with 10 rats in each group: Machined Surface Group (n = 10), Sandblasted Large Acid Grid (SLA) Surface Group (n = 10) and Resorbable Blasting Material (RBM) Surface Group (n = 10). Titanium implants were surgically placed in the right tibias of the rats with sterile physiological serum cooling. Immediately before the implants were placed, local enamel matrix protein was applied to the prepared sockets and then the implants were placed. The rats were euthanized after waiting for osseointegration for four weeks and the implants were taken with the surrounding bone tissues after the soft tissues were removed. The bone–implant contact of all implants was analyzed by biomechanical method and recorded in Newton·cm^{-1} (N·cm^{-1}). When the obtained biomechanical data were examined, the average bone–implant contact value was found to be 2.24 ± 0.67 (N·cm^{-1}) in machined surface implants, 4.5 ± 1.36 (N·cm^{-1}) in SLA surface implants and 3.24 ± 0.94 (N·cm^{-1}) in RBM surface implants. A statistically difference was detected between machined surface implants and SLA surface implants (P<0.05; P=0.02). It can be stated that local enamel matrix protein application may increase bone–implant connection in SLA surface implants.

Key words: Local enamel matrix protein; titanium implant; osseointegration; bone implant connection, bone implant contact

RESUMEN

El objetivo de este estudio es investigar los niveles de osteointegración de implantes con diferentes superficies aplicadas localmente con proteína de matriz de esmalte mediante métodos biomecánicos. Se incluyeron en el estudio treinta ratas Spraque Dawley hembras adultas con un peso de 300–350 g como sujetos. Las ratas se dividieron en 3 grupos con 10 ratas en cada grupo: Grupo de superficie mecanizada (n = 10), Grupo de superficie de rejilla ácida grande pulida con chorro de arena (SLA) (n = 10) y Grupo de superficie de material de granallado reabsorbible (RBM) (n = 10). Los implantes de titanio se colocaron quirúrgicamente en las tibias derechas de las ratas con enfriamiento con suero fisiológico estéril. Inmediatamente antes de colocar los implantes, se aplicó proteína de matriz de esmalte local a los alvéolos preparados y luego se colocaron los implantes. Las ratas fueron sacrificadas después de esperar la osteointegración durante cuatro semanas y los implantes se tomaron con los tejidos óseos circundantes después de que se eliminaron los tejidos blandos. El contacto hueso–implante de todos los implantes se analizó mediante un método biomecánico y se registró en Newton·cm^{-1} (N·cm^{-1}). Cuando se examinaron los datos biomecánicos obtenidos, se encontró que el valor promedio de contacto hueso–implante era de 2,24 ± 0,67 (N·cm^{-1}) en implantes de superficie mecanizada, 4,5 ± 1,36 (N·cm^{-1}) en implantes de superficie SLA y 3,24 ± 0,94 (N·cm^{-1}) en implantes de superficie RBM. Se encontró una diferencia estadísticamente significativa entre los implantes de superficie mecanizada y los implantes de superficie SLA (P<0,05; P=0,02). Se puede afirmar que la aplicación local de proteína de matriz de esmalte puede aumentar la conexión hueso–implante en implantes de superficie SLA.

Palabras clave: Proteína de matriz de esmalte local; implante de titanio; osteointegración; conexión hueso–implante, contacto de implante óseo

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INTRODUCTION

'Osteointegration' in dental implants was introduced by Branemark many years ago [1]. Since this situation has emerged, dental implants have been accepted as a successful treatment that replaces the teeth that are not present in the patient. Dental implants are clinically important and the most important benefit that differs from bridge prosthesis is that they eliminate the preparation of healthy teeth required in bridge prosthesis. In addition, as a result of implant application in patients with missing teeth; chewing functions, quality of life and patient comfort become more significant. Due to this situation, dental implants are an important treatment option to restore the functions of missing teeth [2, 3].

In the early period of implant applications, unsuccessful results could be obtained as a result of complications caused by various factors. Examples of these factors are bleeding, infection and pain at the peri–implant tissues. In addition, the lack of initial tightness, infection in the bone around the peri–implant tissues, and the formation of bone defects in the surrounding tissues during implant application are also among the reasons of failure [4, 5, 6]. The reasons for implant failure can be listed as factors related to; the host factors, implant surface, surgical procedures, implant fixture structure, and implant prosthesis. The quality and amount of bone in the area where the implant is applied, initial stability, angles and orientation of the implant, surface roughness, length and diameter of the dental implant, macro structure and microstructure of the implant fixture are key factors for implant success [4, 5, 6, 7].

Emdogain® (EMD, Straumann; Basel, Switzerland) is a xenogenic material that is generally used for regeneration in periodontal tissues [8]. The EMD, an enamel matrix derivative, is a biomaterial containing amelogenins obtained from pigs in the embryological period. Enamel matrix derivative is purified acidic form material. EMD contains enamel matrix proteins which play an important role in tooth root development. EMD stimulates cytokines that act as stimulators in protein synthesis, such as platelet–derived growth factor–AB, BMP–2, BMP–7, vascular endothelial growth factor, fibroblast growth factor–2, osteopontin (OPN), and alkaline phosphatase [8, 9, 10, 11, 12].

Recently, emdogain® has been used for the treatment of periodontal disease with no reported allergic reactions or adverse events. EMD has demonstrated better periodontal clinical parameter improvement, radiographic defect filling, and higher soft tissue density compared to controls in the treatment of intraosseous defects. Most studies have shown that the treatment of infraosseous defects using EMD yields significantly better results compared to open flap surgery alone [8, 9, 10, 11, 12, 13].

Studies have reported that the surface properties of implants are one of the main factors that are important for long–term implant survival [14]. Long–term successful clinical results have been achieved in prosthetic treatments of titanium implants with a machined surface [14]. However, since dental implant supported prosthetic treatment with a machined surface require long–term osteointegration processes and the patient has to wait for a significant time, the properties of the implant surfaces are modified to reduce adverse conditions [14, 15].

The roughening of the titanium surface results in the presence of excellent bone cells on the implant surface. Rough surface implants have better bone cell response when comapared with machined surface. The titanium implant surface is roughened under a certain pressure using materials, both with acid and in combination with particles such as TiO_2, Al_2O_3. While this method changes both the geometric and chemical parameters of the implant surface, it is recognized that such a sprayed or etched surface changes mainly in topographic aspects. Better bone tissue implant surface interaction were reported on rough surfaces compared to machined surfaces [14]. The purpose of the RBM technique is to create a rough area on the implant surface after sandblasting. It has been reported that the bioceramics used in the RBM technique reduce possible biocompatibility problems by embedding particles on the implant surface [14, 15].

The effect of enamel matrix protein on bone regeneration has taken its place in the literature. It is stated that the regeneration mechanism of intra–bone defects and the fracture healing mechanism are quite similar to the osseointegration process of implants. When evaluated from this perspective, it can be thought that local enamel matrix protein application to the implant site may positively affect the osseointegration process of titanium implants. The aim of this study is to evaluate the effects of locally applied enamel matrix protein on osseointegration levels in implants with three different surfaces; sandblasted and large acid grid, resorbable blast material and machined surfeces.

MATERIAL AND METHODS

Study design and animals

Ethics committee approval required for the study was obtained from Firat University, Elazig, Turkiye, Local Experimental Animals Ethics Committee (Meeting date: 16 January 2019, number of meeting: 2019/01, decision number: 04, protocol number: 2017/98). A total of 30 spraque dawley female rats (*Rattus norvegicus*) weighing (Balance Shimadzu, Japan) 400–450 g were used. The rats were randomLy divided into 3 groups. Temperature control was carried out in the area where the rats were located. The study design was determined to include 10 rats in each group. Rats were randomLy divided into 3 groups: Machined Surface Group (n = 10), SLA Surface Group (n = 10), and RBM Surface Group (n = 10). Food and water access of the rats was continuous throughout the experiment period. In addition, a 12 hour day and 12 hour night cycle was provided. Implants with 3 different surface modifications were selected for the experiment. During the implant application, local enamel matrix protein (Emdogain®, Straumann, Basel, Switzerland) material was applied to the implant sockets of all groups.

Surgical Procedure

There was no deprivation of any additional nutrients or conditions before, during or after the experiment. Before the procedure, all subjects were injected intramuscularly with Ketamine Hydrochloride (Ketamidor–Richte Pharma) 40 $mg \cdot kg^{-1}$ and Xylazine (Rompun–Bayer) 5 $mg \cdot kg^{-1}$ for anesthesia, following the rules of asepsis and antisepsis. The operation area was washed with povidone iodine solution. After washing, it was covered with sterile drapes and the operation area was left open to perform surgical

procedures. To achieving hemostasis locally 0,5 mL, 4% Articaine containing 0,006 mg·mL^{-1} Epinephrine (Ultracain DS – Aventis, France) was injected to the operation area. After a skin incision was made on the tibias of the rats used in the study, the muscle and soft tissues were dissected and the metaphyseal parts of the tibias were exposed. One standard implant cavity was craeted surgivally in the right tibia of each rat with the drills. The implant sites were created sequentially, first with a point drill, then with a 1.8 mm diameter pilot drill, and finally with a 2.5 mm diameter final drill. Titanium implants (Implance Implant Systems, AGS Medical Corporation, Istanbul, Türkiye) were placed in each cavity at the bone level (FIG. 1).

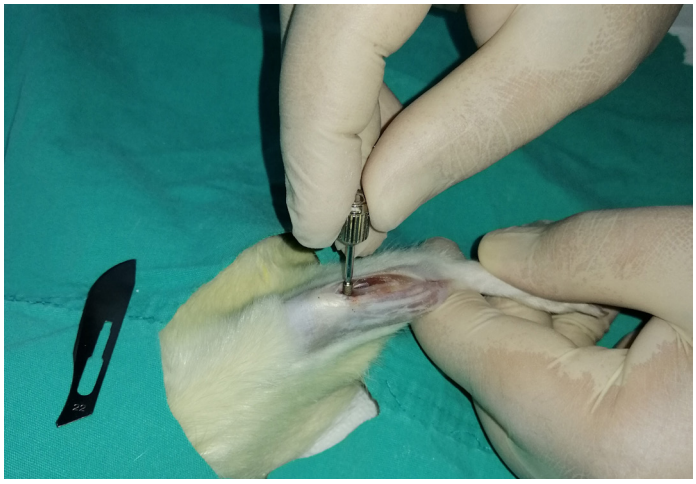

FIGURE 1. Integration of implant in corticocancellous bone tissue in the metaphyseal part of the rats tibia bone after surgical elevation of the soft tissues

The tibial skin and soft tissues was then sutured with 4/0 polyglactin absorbable sutures after restored to their original positions. After surgical interventions, cefazolin sodium (50 mg·kg^{-1}) as an antibiotic was administrated and Tramadol Hydrochloride (0,1 mg·kg^{-1}) was administrated intramuscularly as an analgesic for infection and pain control.

Biomechanical analysis

After four weeks experimental period the rats were sacrified and evaluation of implant osseintegration; bone implant connection (BIC) was performed using the biomechanical method. After obtaining the samples implants and surrounded bone tissues (FIG. 2), they were stored in 10% Formaldehyde for analysis.

Evaluation was done immediately in order to prevent fluid loss in the tissues. For analysis, the samples were adapted to the experimental setup as polymethylmethacrylate blocks, then biomechanical measurements were made using a reverse torque device (Mark–10, MTT01–12, Cap Torque Tester, USA). Force was applied to the manual until resistance was lost from the implants and the value obtained on the digital device screen was determined (N·cm^{-1}) (FIG. 3).

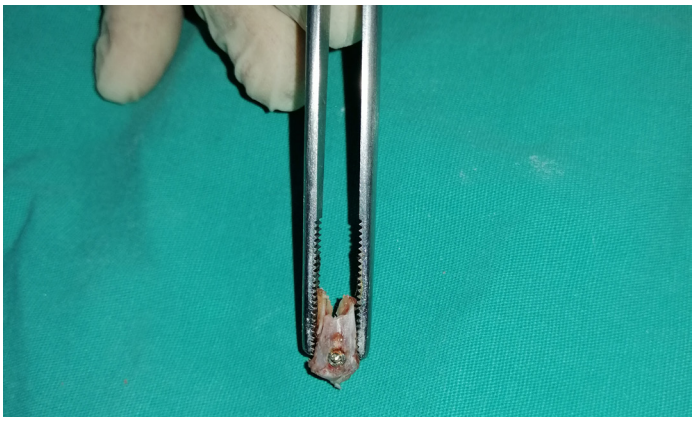

FIGURE 2. Collecting the implant and surrounding rats bone tissues from the surrounding soft tissues for biomechanical analysis

FIGURE 3. Biomechanic bone implant connection analysis of the implants with reverse torque

Statistical analysis

The biomechanical bone implant cojnnection data obtained as a result of the tests were analyzed with the SPSS 22 package program (IBM). Kolmogorov–Smirnov and Shapiro–Wilk tests were used to determine whether the data were normally distributed. Since it was determined that the data did not conform to a normal distribution, nonparametric statistical methods were used. Kruskall–Wallis test was used to determine whether there was a difference between the groups. Mann Whitney U test was used in pairwise comparisons to determine the group causing the difference. The significance level of the statistical results obtained from all data was taken as $P<0.05$

RESULTS AND DISCUSSION

Inappropriate samples were excluded from the study. Analysis was performed with 7 samples in each group. As a result of the analyses, the mean BIC value on the machined surface was 2.24 ± 0.67 N·cm^{-1}, while 4.5 ± 1.36 N·cm^{-1} on the SLA surface and 3.24 ± 0.94 N·cm^{-1} on the RBM surface. As a result of the statistical analysis, significant difference was detected between the groups (*P*=0.003), a statistically difference was detected between the machined and the SLA surface implants (*P*=0.02) (TABLE I). In SLA surface implants biomechanical BIC levels detected highly compared with machined surface implants.

Emdogain has been produced as a biological agent that induces regeneration in periodontal defects. Emdogain is produced from amelogenins and originates from pig embryo tissue [8, 9, 10, 11, 12]. *In vivo* and *in vitro* studies have proven the effect of enamel matrix derivatives on ligament cells in periodontal tissues. It has been reported enamel matrix derivates play an dominant role in the development of the attachment apparatus during cementogenesis. It's reported that emdogain can be clinically safely used. In this study, the effect of emdogain use on dental implants with different surface structures on implant osseintegration was evaluated on rats [8, 9, 10, 11, 12].

TABLE I
Biomechanic bone implant connection (BIC) levels (N·cm) of the groups

Parameter	Groups	N	Mean	Standard Deviation	Min.	Max.	*P**
BIC	Machined	7	2.24	0.68	1.1	3.1	<0.05
	RBM	7	3.24	0.95	1.7	4.5	
	SLA[a]	7	4.50	1.37	2.4	6.8	

*: Kruskal Wallis, *P*=0,003. Mann Whitney U, *P*=0,002. [a] Statistically different compared with the machined surfaced implants. RBM: Resorbable Blast Material, SLA: Sand blasted large acid grid

Enamel matrix protein supports the regeneration of the periodontium by preventing epithelial infiltration. EMD induces angiogenesis of human microvascular cells [8, 9, 10, 12] In one study, it was histologically proven that periodontal regeneration occurs using EMD [16]. Boyan *et al*. [17], found that adding 4 mg of EMD to demineralized freeze–dried bone allograft (DFDBA) increased the regeneration of the bone tissues when compared with DFDBA alone. Rosen *et al*. [18] demonstrate the clinical benefits of using a combined treatment technique when EMD is used with DFDBA or freeze–dried bone allograft (FDBA). Harrel *et al*. [19], successfully used DFDBA mixed with EMD with minimally invasive periodontal surgical application to treat 130 periodontal defects.

Hoidal *et al*. [20] found that adding EMD to DFDBA did not achieve a better improvement in soft and hard tissue parameters measured 6 months after surgery compared to DFDBA alone. In 2002, Velasquez–Plata *et al.* [21] compared the use of EMD alone or combination with xenograft and they reported no significant difference between the groups in terms of PD reduction or CAL gain. As well as, Lekovic *et al.* [22], showed a increasing periodontal treatment parameters, and bone filling with the combination of xenograft and EMD compared to EMD alone. In study on dogs, it has been reported that EMD has positive effects around the implant in guided bone regeneration [23].

In this study, the effect of emdogain on various implant surfaces was evaluated. The osteointegration of implants is directly related to the surface structure. Researchers compared the survival rate of machined and double etched implants in one study and they reported that machined implants had a higher failure rate in areas with poor bone quality than double etched implants [24]. Stach and Kohles [25], obtained similar results in their study. In a 19–year retrospective study, it was reported that failure rates decreased when the roughness of the surfaces increased [26].

In a short–term observational study of implants applied in the anterior region, the survival rate of RBM implants was reported to be 90% [27]. In another study, SLA–surfaced implants and RBM–surfaced implants were compared. In cases of poor bone quality low rate have been found in the survival rate of implants with RBM surface [28]. In our study SLA surface implants shown better biomechanic BIC when compared with machined surface implants. Özcan *et al*. [5], evaluated the biomechanical osseointegration levels of SLA, RBM and machined surface implants with allogeneic bone transplantation in rats and they reported that SLA surface implants have better osseointegration comapared with RBM and machined implants; I also reported that SLA surface implants showed better biomechanical osseointegration; BIC, level than RBM and machined surface implants. Bingül *et al*. [4], in their four–week study, examined the osseointegration values of SLA, RBM and machined surface implants to which they applied local zoledronic acid biomechanically. The researchers reported that osseointegration values were statistically higher in SLA and RBM implants compared to machined surface implants in both experimental and control groups. In addition, it was determined that biomechanical osseointegration levels were higher in SLA group implants to which local zoledronic acid was applied than in RBM surface implants. However, according to the researchers' results, osseointegration values of RBM surface implants were determined to be higher than SLA surface implants in subjects to whom local zoledronic acid was not applied. Based on the results of Bingül *et al*. it can be stated that SLA surface implants are more advantageous in local biomimetic applications in this study [4].

This study has some limitations. First, the mechanism of implant osseointegration with enamel matrix protein could not be investigated molecularly due to the method used in this study. Second, although in vivo studies are vital for understanding the implant–bone tissue relationship, the data obtained from these studies can only be used to estimate the corresponding pathways in humans. Third, we could not evaluate the bone–implant fusion of titanium implants in the long term in this study. Fourth, while long bones such as tibia and femur ossify endochondral, jaw bones (mandible–maxilla) ossify intramembranously, therefore they have different osteogenic properties and therefore may have responded differently to local emdogain application. In addition, the small sample size used in the analysis phase of the study may limit the generalization of the obtained results [29].

CONCLUSION

In this study, emdogain was applied to the sockets of the machined, RBM and SLA surface implants and its effect on osteointegration was evaluated. In the light of the results, similar to previous studies, the effect of emdogain application on osteointegration was found to be statistically significantly higher in implants with SLA surfaces compared to machine–made implants. In this case, it is seen that the surface roughness of the implants increases the rate of osteointegration. In order to obtain more definitive results; more advanced studies including histopathological, immunohistochemical and molecular analyses are needed.

Conflict of interests

The authors have no conflict of interests to declare concerning the authorship or publication of this article.

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