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Research Article

Histological and scanning electron microscopy investigation of the effects of titanium surface modifications on osseointegration in rabbits

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ABSTRACT

Objective: This study aimed to compare the novel Estaş Medical Anodization (EMA) surface treatment technique with the techniques commonly used in the literature and to examine their effects on osteointegration in the rabbit tibia.

Methods: A total of 24 rabbits used in this study were divided into 3 groups, with 8 rabbits in each group. Using both tibias of all rabbits in the study, screws belonging to the control group were placed in the left tibia, and the right tibia belonging to the experimental group were placed. In the first 8 rabbits, a single experimental group in the right tibia were used; in the second 8 rabbits, 2 different experimental groups in the right tibia were used; and in the last 8 rabbits, 2 different experimental groups in the right tibia were used. Thus, 5 different experimental groups with 8 screws in each group and a separate control group were formed for each of them. EMA-treated surfaces were named 200-800 nm iris oxidation and 800-1200 nm gray oxidation according to the TiO_2 layer thickness. Group 1 was implanted with mini-screws prepared with chemical etching+EMA iris oxidation, while group 2 was implanted with sandblasted, large-grit and acid-etched (SLA) mini screws treated with EMA gray oxidation. Group 3 was implanted with mini-screws treated with EMA gray oxidation, group 4 was implanted with mini-screws treated with chemical etching+micro-arc oxidation, and group 5 was implanted with mini-screws treated with chemical etching+EMA gray oxidation. The control group was implanted with mini-screws prepared with pure titanium. At the end of 6 weeks, osseointegration percentages were calculated and compared using histological and scanning electron microscope (SEM) analyses.

Results: The histological results confirmed the increase in osseointegration percentages in all experimental groups compared to those that received pure titanium implants (P values control group vs group 1=.005, control group vs group 2, 3, 4, 5=.001). The comparison between the groups revealed that the chemical etching+EMA gray oxidation modification technique (group 5) significantly increased osseointegration compared to the SLA+EMA gray oxidation technique (group 5 vs group 2 P=.016) and the chemical etching+EMA iris oxidation technique (group 5 vs group 1 Pp=.001). The EMA gray oxidation technique (group 3) significantly increased osseointegration compared to the chemical etching+EMA iris oxidation technique (group 1) (group 3 vs group 1 P=.043). The results of the SEM analysis showed that osseointegration was significantly increased in all experimental groups compared to that in the pure titanium (control) group (P values control group vs group 1, 2, 3=.001, control group vs Group 4,5=.006). The mean osseointegration percentage in the experimental groups was the highest in group 5, followed by group 4, group 3 and group 1 equally, and group 2. However, the differences among the experimental were not significant (group 1, group 2, group 3, group 4 vs group 5 P=.408).

Conclusion: The EMA titanium surface modification techniques we developed significantly increased osseointegration compared to the pure titanium surface. The EMA gray oxidation technique seems to result in higher osseointegration rates than the EMA iris oxidation technique, and similar rates can be found with the SLA and chemical etching techniques.

Level of Evidence: N/A.

Introduction

Osseointegration, as a medical term, refers to the biological, structural, and functional interaction between the living bone and the load-bearing implant,[1] while clinically it refers to the connection of the bone tissue with the implant, thus increasing the survival of the implant under physiological loads.[2] The adhesion of the bone to the implant and the long-term retention of this adhesion are essential for successful clinical results.[3]

Mechanical retention and osseointegration at the interface between the mini-screw and the bone play a key role in stability.[4] Many surface modification techniques have been described in an attempt to increase the osteoconductivity of mini-screws and provide stability.[5] Surface treatments create a bioactive medium on the screw surface. Surface roughness activates osteoblast proliferation and migration of osteoblasts to the screw-bone interface.[6] The sandblasted, large-grit and acid-etched (SLA) surface treatment technique, has been reported to be a safe method with predictable results.[7,8] SLA surfaces have higher bone-implant contact (BIC) ratios than titanium surfaces.[9] Although the positive effects of rough surfaces are well known, the optimal surface modification technique for the adsorption of osteoblastic cells and rapid osteointegration response has not been reported yet.[5,10]

As far as we know, the literature holds many studies on the mini-screw surfaces prepared by SLA or chemical etching processes but lacks a study that combines these surface modification techniques. The current study aimed to compare the novel Estaş Medical Anodization (EMA) surface treatment technique with

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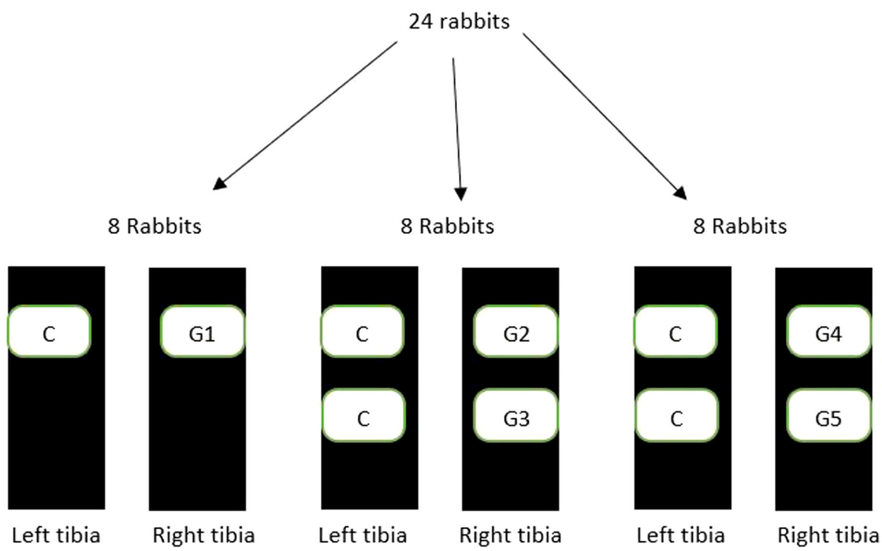

Figure 1. Creation of working groups.

the techniques commonly used in the literature and to examine their effects on osteointegration.

Material and methods

Design and experimental procedures

This study was approved by the Sivas Cumhuriyet University Animal Experiments Local Ethics Committee, number 65202830-050.04.04 487. A total of 24 New Zealand white rabbits, aged 6 to 8 months and weighing 3,000 to 3,200 grams, were used in the study. All rabbits underwent an adaptation period of approximately 2 weeks. These rabbits are divided into 3, with 8 rabbits in each group among themselves. Using both tibias of all the rabbits in the study, screws belonging to the control group were placed on the left tibias and the right tibias belonging to the experimental group were placed. In the first 8 rabbits, 8 screws belonging to a single different experimental group in the right tibia and 8 screw surfaces belonging to the control groups were used in the left tibia. In the second 8 rabbits, a total of 16 screws belonging to 2 different experimental groups in the right tibia and 16 screws belonging to the control group were placed in the left tibia. In the last 8 rabbits, a total of 16 screws belonging to 2 different experimental groups were placed in the right tibia and 16 screws belonging to the control group were placed in the left tibia (Figure 1). While the region of the bone in the proximal diaphysis is used in rabbits where a single type of screw is placed, in rabbits where 2 types of screws are placed, one of the screws is placed in the proximal diaphyse zone and the other screw is 20 mm distal. Thus, 5 different experimental groups and a separate control group were created for each of them.

A total of 80 pure titanium conical screws (Estaş Medikal, Sivas, Türkiye), 2.5 × 6 mm in size, were specially prepared for this study, with 8 screws being used in each group. The mini-screws were divided into 5 different groups according to their surface modification properties (Table 1). EMA-treated surfaces were named 200-800 nm iris oxidation and 800-1200 nm gray oxidation according to the TiO_2 layer thickness. Group 1 was implanted with mini-screws prepared with chemical etching +– EMA iris oxidation, while group 2 was implanted with SLA mini screws treated with EMA gray oxidation, Group 3 mini screws treated with EMA gray oxidation, group 4 mini screws treated with chemical etching+micro-arc oxidation, and Group 5 mini screws treated with chemical etching+EMA gray oxidation (Figure 2).

The mini-screw implantation was performed by the same operator under sterile conditions and anesthesia at the Experimental Animal Application and Research Center of the Faculty of Medicine at Erciyes University. In order to increase the cortical bone density in the rabbit's tibia and provide better stability, a hole was drilled with a 2.0 mm drill and mini screws were inserted with a special constant torque (35 Ncm) screwdriver (Figure 3).

During the experiment, 2 rabbits died. Since these rabbits had screws belonging to both the experimental and control group of group 2, 3, 4, and 5 in these rabbits, the work continued with 7 samples in these groups. All animals were sacrificed with high-dose phenobarbital at the postoperative sixth week.[11] Their tibias were excised for histological and scanning electron microscope (SEM) examination.

Histological analysis

Histological analysis was performed at the Research Laboratory of the Faculty of Medicine at Erciyes University (Kayseri, Türkiye) and the Department of Medical Pathology of the Faculty of Medicine at Erciyes University (Sivas, Türkiye). Transverse tibial sections, including the screws, were taken from all rabbits in the experimental and

HIGHLIGHTS

- Novel EMA surface modification technique increases osseointegration compared to the pure titanium surface.
- EMA gray oxidation results in higher osseointegration rates compared to EMA iris oxidation.
- The EMA gray oxidation technique yields similar osseointegration rates with the widely used SLA technique and chemical etching technique.

Table 1. Surface modifications used in the experimental and control groups.

Groups	Left tibia	Right tibia
Group 1	Titanium	Chemical etching+EMA iris oxidation
Group 2	Titanium	SLA+EMA gray oxidation
Group 3	Titanium	EMA gray oxidation
Group 4	Titanium	Chemical etching+micro-arc oxidation
Group 5	Titanium	Chemical etching+EMA gray oxidation

EMA, Estaş medical anodization; SLA, sandblasted, large-grit and acid-etched.

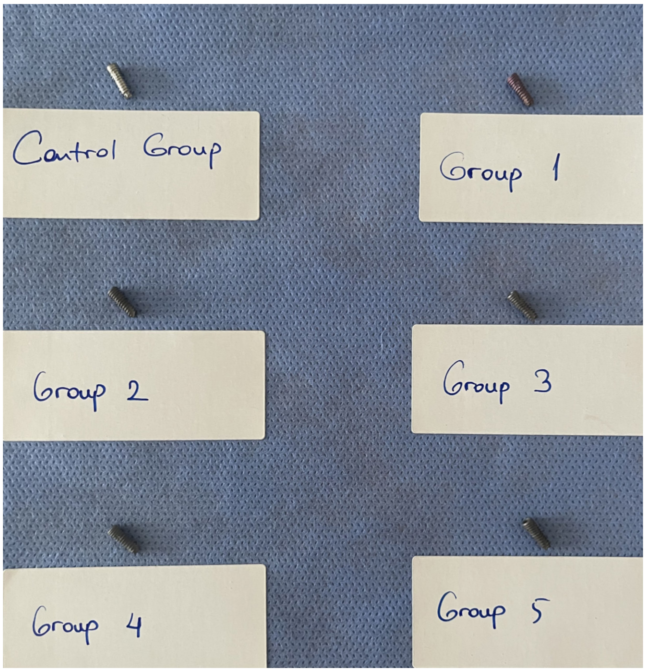

Figure 2. Titanium screws used in experimental groups and control groups.

control groups. Histomorphological staining was done using hematoxylin-eosin (Figure 4). The BIC ratios were calculated by dividing the thread surface length with the surrounding bone tissue by the total thread surface length (Figure 5).[12]

Scanning electron microscope and energy-dispersive x-ray spectroscopy analyses

Scanning electron microscope analysis was performed at the Technology Research and Application Center at Erciyes University (Kayseri, Türkiye). The SEM images were recorded for evaluation of surface topography and composition. (Figure 6) In addition, EDS analysis was performed to determine the mini-screw surface composition and new bone formation areas. The BIC ratios were calculated in the same way as in the histological examination.[12]

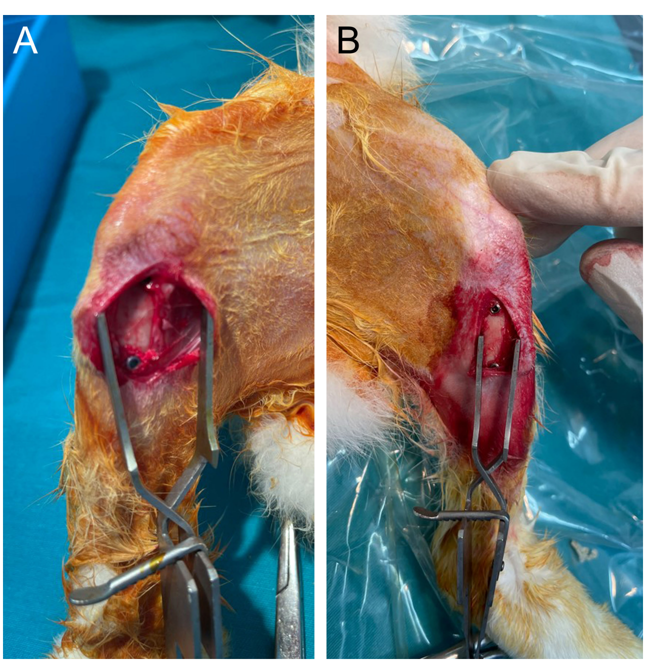

Figure 3. Intraoperative images of experimental group (A) and control group (B), with the screws placed in rabbits' tibias.

Statistical analysis

Statistical analyses were performed using the SPSS v.20.0 for Windows software (IBM SPSS Corp.; Armonk, NY, USA). The Shapiro–Wilk test was performed on all variables to test the normal distribution. The *t*-test was used when comparing a variable in 2 independent groups, while 1-way ANOVA was used when comparing variables obtained from more than 2 independent groups. When comparing 2 dependent measurement values, the paired samples *t*-test was utilized and when comparing more than 2 dependent measurements, ANOVA and Bonferroni test were used for repeated measurements.

Results

Findings from scanning electron microscope and energy-dispersive x-ray spectroscopy analyses

The findings from the SEM analysis confirmed that all mini-screws had homogeneous and acceptable surfaces, with no defects such as pores and craters on the surfaces. The elemental composition of the screw surfaces was similar in all screws. We also observed that there was no dissociation at the implant-bone interface in all groups. The EDS analysis confirmed the titanium, aluminum, and vanadium peaks on the mini-screw surfaces (Figure 7). Again with the EDS analysis, oxygen, calcium, phosphorus, and carbon peaks were observed in the new bone formation areas; these regions were compatible with the bone matrix (Figure 7).

A comparison of the experimental groups showed that all surface modification techniques increased osseointegration significantly compared to pure titanium surface (Table 2; *P* values control group vs Group 1,2,3 = .001, control group vs Group 4,5 = .006), while the difference among the groups was insignificant (Table 3; Group 1, Group 2, Group 3, Group 4 vs Group 5 *P* values = .408).

Findings from the histomorphometric analysis

The results of the histomorphometric examination confirmed that all surface modification techniques significantly increased osseointegration compared to pure titanium surface (Table 4; *P* values control group vs group 1 = .005, control group vs group 2,3,4,5 = .001). A comparison of the experimental groups showed that osseointegration was significantly increased in group 5 compared to group 2 (group 5 vs group 2 *P* = .016) and group 1 (group 5 vs group 1 *P* = .001) and in group 3 compared to group 1 (group 3 vs group 1 *P* = .043) (Table 5). Group 3 (group 3 vs group 5 *P* = .665) and group 4 (group 3 vs group 5 P = .582) did not differ statistically significantly from group 5.

Discussion

In studies where various surface modification techniques have been examined to increase the clinical success of mini-screws, it has been shown that rough surfaces provide faster healing and better stability.[13] Among the numerous known techniques, it is a known fact that mini-screw surfaces prepared with the SLA and chemical etching process are more successful.[14] The reason for this can be the homogeneous surface roughness created by sandblasting and acid-etching processes and allowing more cell adhesion than the treated surfaces.[15] While the literature holds studies in which a surface modification technique is compared with a pure titanium surface, the current

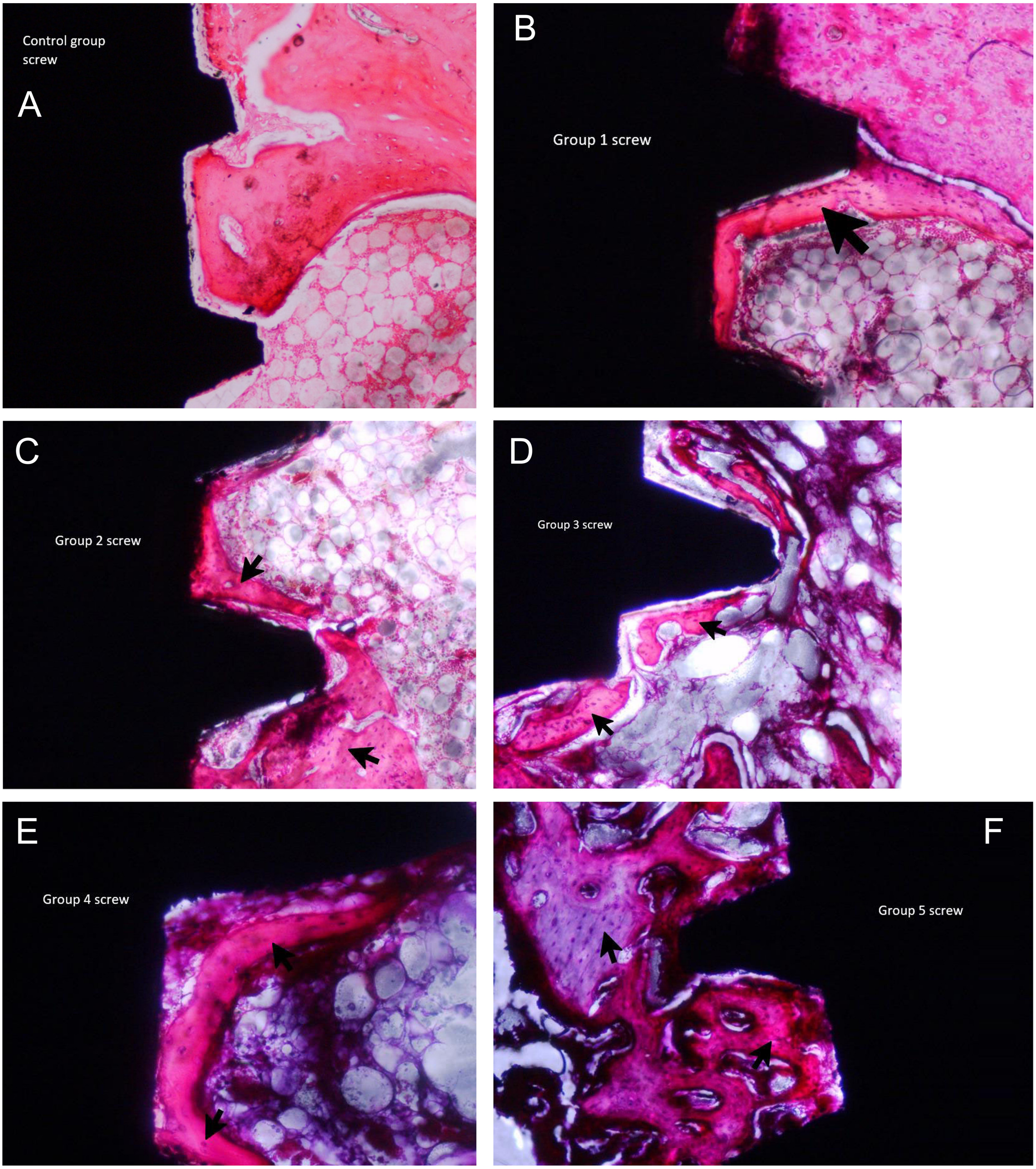

Figure 4. Control group (A), group 1 (B), group 2 (C), group 3 (D), group 4 (E), and group 5 (F) histological images obtained from the experimental and control groups.

study may also be considered an important one in terms of comparing 5 different surface modification techniques with each other and with the pure titanium surface. In addition, a new surface modification technique has been introduced to the literature in terms of mechanics, corrosion, and biocompatibility on the titanium surface.

In our study, we focused on the degree of contact of the screws with the bone tissue. For histological examination, a section was taken together with the screw, as screw removal may have adversely affected osseointegration. The osseointegration rate on the mini-screw surface (BIC ratio) was found by calculating the whole size of the screw in the bone and the new bone areas around the screw.[12] Mechanical adhesion, osseointegration, and good primary stability were shown to be important factors for a high BIC ratio.[16] Time to healing after implantation is as important as the surface properties of the mini-screws for stability. A healing period of over 4 weeks was

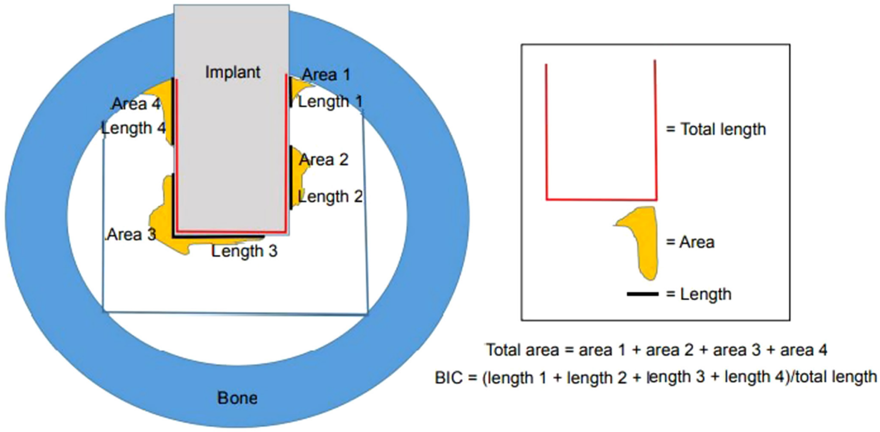

Figure 5. Calculation method of BIC percentages.

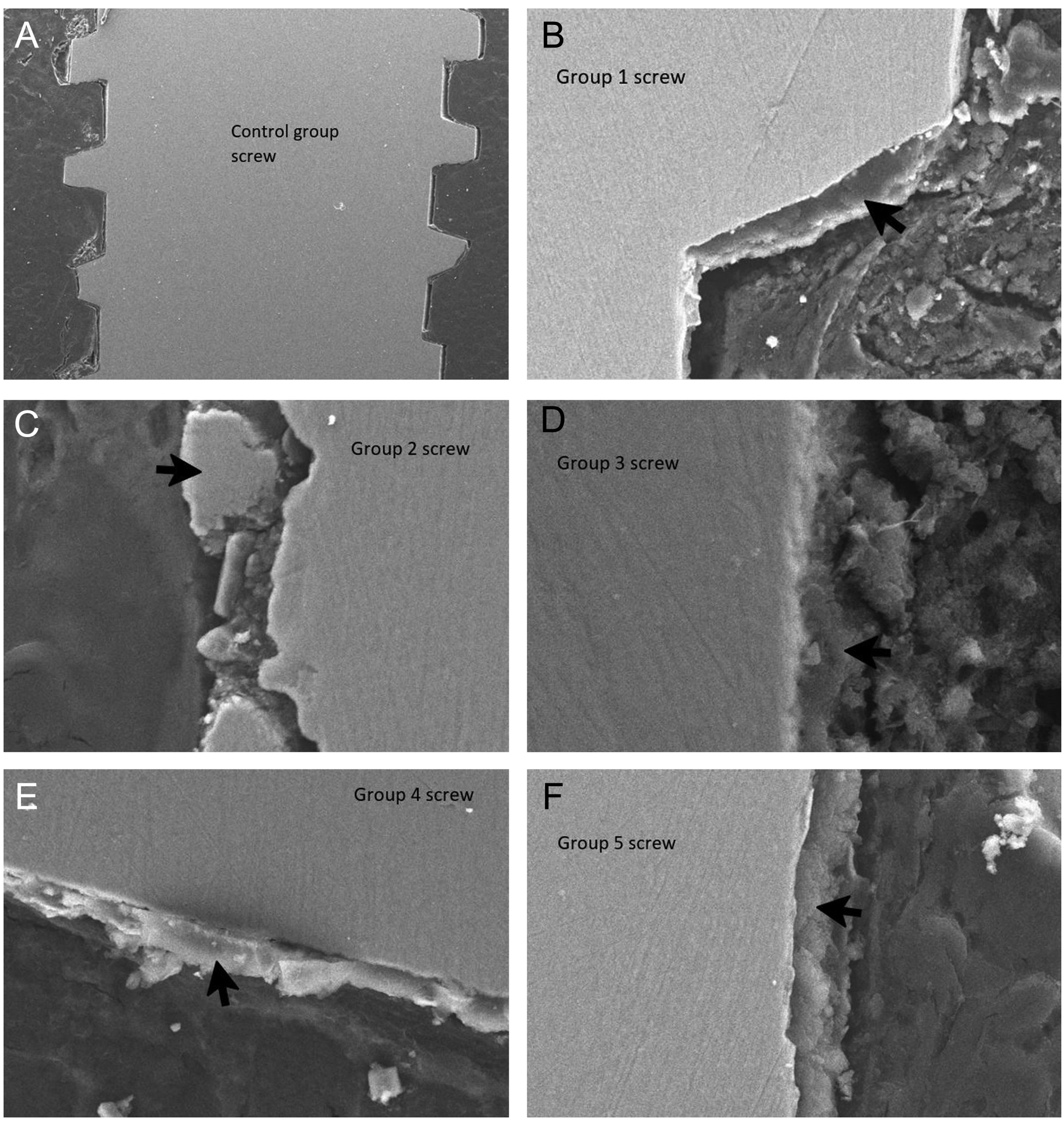

Figure 6. Control group (A), group 1 (B), group 2 (C), group 3 (D), group 4 (E) and group 5 (F) scanning electron microscope images obtained from the experimental and control groups.

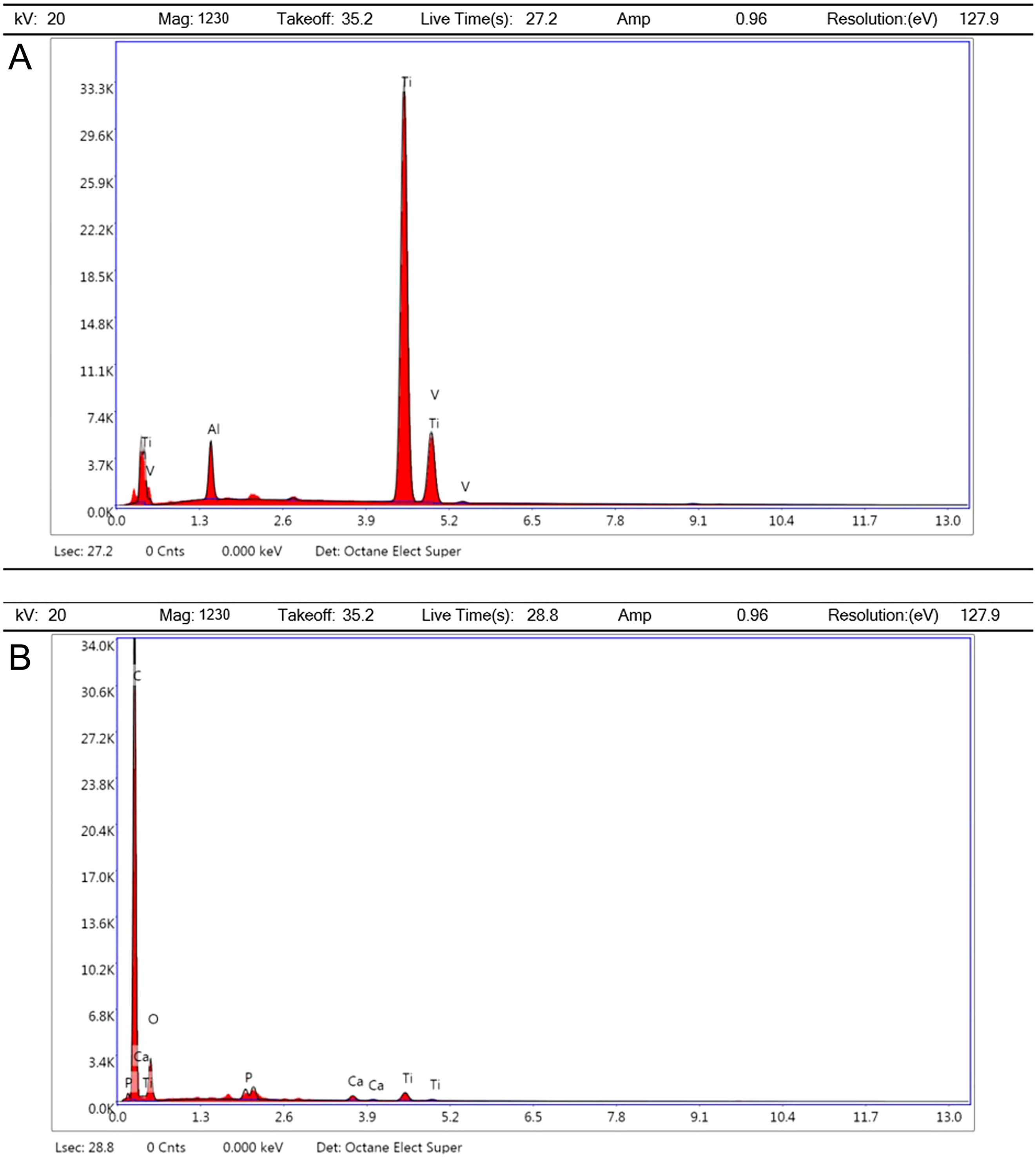

Figure 7. EDS analysis of the screw surface (A) and new bone formation areas (B).

reported to have significant effects on new bone formation and stability.[11] In the current study, osseointegration was evaluated at the end of 6 weeks in line with the literature.

Implants with surface modifications provide faster healing and better stability with an increase in the surface area contacting the bone.[13] There have been many studies on surface modifications in the literature which have shown the highest bone formation rate is achieved with the use of implants with an SLA surface.[13] In our study, we also noted that SLA surfaces increased osseointegration; however, implants with the recently defined EMA surface modification technique also provided osseointegration at similar rates to implants with an SLA surface alone.

Several studies have reported osseointegration rates ranging from 10% to 58% around the mini-screw, however, it is not clear from these reports what the optimal osseointegration rate should be to ensure adequate stability.[17] In another study, a BIC of 42% was shown to be

Table 2. Comparison of the experimental groups with the control groups in terms of scanning electron microscope

	Number of Evaluated Screws	BIC Ratio	Standard Deviation	*P*
Group 1	8	0.361	0.156	**.001**
Control group	8	0.100	0.050	
Group 2	7	0.345	0.076	**.001**
Control group	7	0.112	0.046	
Group 3	7	0.362	0.107	**.001**
Control group	7	0.112	0.046	
Group 4	7	0.417	0.182	**.006**
Control group	7	0.150	0.058	
Group 5	7	0.495	0.237	**.006**
Control group	7	0.150	0.058	

Significant *P* values are highlighted in bold.

Table 3. Intergroup comparison of the experimental groups in terms of scanning electron microscope

Groups	Number of evaluated screws	BIC ratio	Standard deviation	*P*
Group 1	8	0.360	0.150	
Group 2	7	0.340	0.070	
Group 3	7	0.360	0.100	.408
Group 4	7	0.410	0.180	
Group 5	7	0.490	0.230	

Table 4. Comparison of the experimental and control groups in terms of histomorphometric examination

	Number of evaluated screws	BIC ratio	Standard deviation	*P*
Group 1	8	0.162	0.051	**.005**
Control group	8	0.061	0.043	
Group 2	7	0.207	0.097	**.001**
Control group	7	0.064	0.045	
Group 3	7	0.314	0.124	**.001**
Control group	7	0.064	0.045	
Group 4	7	0.307	0.105	**0.001**
Control group	7	0.114	0.047	
Group 5	7	0.385	0.106	**0.001**
Control group	7	0.114	0.047	

Significant *P* values are highlighted in bold.

important to establish and maintain osseointegration.[18] In our study, we noticed osseointegration percentages varying between 16% to 38% as a result of the histological analysis and between 34% to 49% as a result of the SEM analysis.

The surface properties of the mini-screw and the new bone tissue formed on the surface were revealed by the SEM and EDS analyses. It has been shown that the titanium surface can have a thin layer of oxygen (TiO_2), and an elemental composition of sodium, calcium, phosphorus, nitrogen, sulfur, chlorine, and silicon in small amounts.[19] It has been reported that mini-screws have standard surface properties that will not adversely affect osseointegration, whereas heterogeneous surface properties can adversely affect osseointegration.[20] In the current study, implants with homogeneous surface properties were used. It has been also reported that mini-screws with medium surface roughness affect osseointegration positively.[21] All mini-screws used in our study were confirmed to have medium surface roughness as a result of the analyses.

Our study had some limitations. The most important limitation stands out as the difference between human and rabbit bone microstructure and metabolism. Since rabbit metabolism is 3 times faster than human metabolism, this information should be considered in

Table 5. Comparison of each experimental group with the other experimental groups in terms of histomorphometric examination

Groups		Mean	Standard deviation	*P*
Group 1	Group 2	−.04464	0.051	.906
	Group 3	−.15179	0.051	**.043**
	Group 4	−.14464	0.051	.060
	Group 5	−.22321	0.051	**.001**
Group 2	Group 1	.04464	0.051	.906
	Group 3	−.10714	0.053	.280
	Group 4	−.10000	0.053	.346
	Group 5	−.17857	0.053	**.016**
Group 3	Group 1	.15179	0.051	**.043**
	Group 2	.10714	0.053	.280
	Group 4	.00714	0.053	1.000
	Group 5	−.07143	0.053	.665
Group 4	Group 1	.14464	0.051	.060
	Group 2	.10000	0.053	.346
	Group 3	−.00714	0.053	1.000
	Group 5	−.07857	0.053	.582
Group 5	Group 1	.22321	0.051	**.001**
	Group 2	.17857	0.053	**.016**
	Group 3	.07143	0.053	.665
	Group 4	.07857	0.053	.582

Significant *P* values are highlighted in bold.

the clinical evaluation of our results.[22] In addition, the results from animal studies may not always be a predictor for human reactions. In addition, the number of subjects was kept as low as possible to minimize the number of sacrificed animals in our study.

In conclusion, the EMA titanium surface modification techniques we developed significantly increased osseointegration compared to the pure titanium surface. The EMA gray oxidation technique seems to result in higher osseointegration rates than the EMA iris oxidation technique and similar rates with the SLA and chemical etching techniques.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: This study was approved by Ethics Committee of Sivas Cumhuriyet University (approval number: 487; date: 25/01/2021).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

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