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A New Method of Detecting Submerged Implants: An Animal Experiment

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Abstract

Objective: There are difficulties in determining the location of submerged implants when cover screws and healing screws are to be replaced. Because of this, a new implant cover screw has been designed. The purpose of this study was to investigate the properties of a novel implant cover screws in rabbits.

Methods: 10 New Zealand White rabbits were randomly divided into two groups. Diastema regions behind the incisor teeth were used for the placement of cover screws. In the control group, the screws (n=20) that received no processing were placed whereas, in the experimental group, the screws (n=20) that top surfaces were coated with europium and dysprosium doped strontium aluminate were placed to the diastema regions. Animals were sacrificed after 6 weeks. Dental LED curing light was applied to the oral mucosa regions in which screws were placed in the experimental group just after sacrification and the visibility of the screws was evaluated. To determine the biocompatibility of the coated screws, oral mucosas which contacted with the screws, livers and kidneys were removed and examined histopathologically.

Results: After light application, only the screws in the upper jaws of the experimental group became visible (n=10). Histopathological examinations performed on the kidneys, livers and oral mucosa tissues which contacted with the screws. There were no significant differences between the experimental and control groups regarding these tissues.

Conclusion: According to the results of this study, it can be concluded that the titanium implant cover screws coated with europium and dysprosium doped strontium aluminate were biocompatible for rabbits.

Keywords: Biocompatibility; Cover screw; Dental implant; Rabbit; Strontium aluminate

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INTRODUCTION

Nowadays, the best material for the treatment of edentulousness is titanium dental implants. These are surgically implanted in jaws, and then dental prostheses are prepared and placed on them.

Dental implant surgery is performed in two ways, either one- or two-stage surgery. In one-stage dental implant surgery, the body of the implant is placed in the jawbone. A healing screw is screwed into the implant body for healing soft tissue around the implant in the same session. The surgical site is sutured, and the healing screw remains open to the mouth. Therefore, they are called “non-submerged” or “transmucosal” implants. Immediately or after osseointegration, prosthetic stages can take place.

In two-stage dental implant surgery, the body of the implant is closed with a cover screw made of titanium to protect the internal structure of the implant from blood, saliva, and soft and hard tissues until the prosthetic stages after implantation. The surgical site is sutured, and the implant remains completely under the oral mucosa. Therefore, they are called “submerged” implants. After osseointegration, the oral mucosa on the implant is incised or excised during a second surgery. The cover screw is removed and replaced with the healing screw. Then, in the same way as in one-stage surgery, prosthetic stages are started.

Many problems can be encountered when replacing the cover screw with the healing screw. Failure to detect the exact location of the implant may result in more incisions than are necessary. To remove a cover screw of about 3mm in diameter, a region of the required size for implant surgery can be incised, but this situation has led to many complications.

Phosphorescence is a phenomenon whereby a material receives energy from ultraviolet, visible or infrared rays and gives this energy off in its environment for a certain period of time, even after the excitation irradiation ends. Europium and dysprosium doped strontium aluminate ($SrAl_2O_4$: Eu^{+2}, Dy^{+3}) is a pigment that has this characteristic (1). Previous studies have shown that it is biocompatible (2,3) and produces very strong visible light (4).

Coating titanium cover screws with this biocompatible pigment can help the surgeon easily locate implant sites in the mouth. For this purpose, dental LED curing lights which are very powerful light sources and used in nearly all dental clinics can be used. After these light sources are applied in the mouth, the cover screws may become visible under the oral mucosa due to their phosphorescent properties, and the exact location of the implant can be found. In this way, unnecessary incisions can be avoided, and complications can be reduced.

Material that is being considered for use in human bodies is usually tried in animals first. This is also the case for implants. The first

implant studies were performed on experimental animals (5). Therefore, it was decided to use the rabbits for our study. Dental implants were not used. However, screws made from titanium, such as dental implant material, can be considered miniature implants. In addition, the visibility of the screws must be evaluated in live tissues to which blood flow continues. To determine the visibility and biocompatibility of the screws in the oral tissues, it was planned that the screws are placed in jawbones.

The aim of this study is to investigate whether europium and dysprosium doped strontium aluminate coated titanium screws will become visible under the rabbit's oral mucosa after light application and whether they are biocompatible for rabbits.

METHODS

The study was performed at the Ondokuz Mayıs University Faculty of Dentistry in Samsun, Türkiye.

Animals

10 healthy male New Zealand White rabbits were used with a minimum age of 6 weeks and a weight of at least 2kg. Experimental animals were procured from Ondokuz Mayıs University Medico-Surgical Research Laboratory. The number of rabbits was determined by reference to the different implant studies (6,7). Animals were randomly chosen from the supplier and randomly divided into two groups as experimental (n=5) and control (n=5).

In the experimental group, two of the animals died, one on the first day due to nutritional deficiency and the second on the seventh day due to oral infection after the surgery. This situation was diagnosed by the responsible veterinarian in the Ondokuz Mayıs University Medico-Surgical Research Laboratory and reported to the Ondokuz Mayıs University Animal Care and Ethics Committee. After that, permission was given again, and 2 rabbits were treated with the same procedure. As a result, a total of 12 animals was used in the study.

Screws and Their Features

Titanium dental implant cover screws were chosen for the study. The length of the screws was measured as 6mm. The largest diameter at the top surface was 3mm and the smallest diameter at the bottom was 1mm. These screws were used directly in the control group (Figure 1a). On the other hand, screws were used in the experimental group that the top surface of the screw was coated with a long persistent phosphorescent pigment, europium and dysprosium doped strontium aluminate (Figure 1b). All screws were sterilized in a dental autoclave before surgery (Nüve NC 23B, Nüve Laboratory and Sterilization Tech., Türkiye).

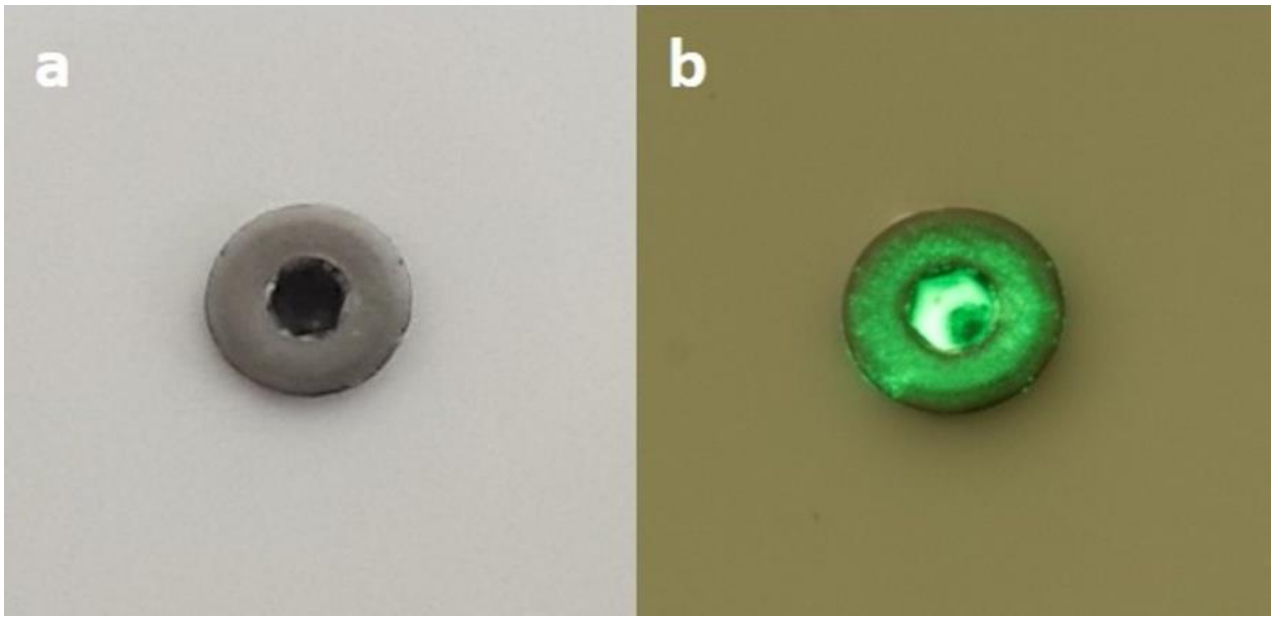

Figure 1. a: The screws that used in the control group without strontium aluminate, **b:** The strontium aluminate coated screws that used in the experimental group.

Presurgical Cadaveric Examination

The screws were planned to be placed on jawbones to examine the effects on oral tissues. For a clear understanding of the anatomy of rabbits' jaws, a mature male rabbit cadaver was examined before surgical implantation of the screws.

Firstly, it was determined that the screws could be placed in the large diastema region between the incisor and cheek teeth. The diastema regions were examined with elevating flap from the upper and lower jaws. The mental foramen was close to the cheek teeth. After that, the upper and lower jaws were cut out coronally, including the cheek teeth.

The jaws were separated from the midline. Radiographs were taken from the right sides. The radiographs showed that the incisor teeth would be damaged if the screws were placed vertically. Therefore, it was decided to place the screws buccolingually.

On the left side of the jaws 1cm behind the incisor teeth, the bones were drilled using a surgical fissure bur with a diameter of 1mm, the same as the screws' groove diameter. Then, radiographs were taken, and no damage was detected on the incisor teeth.

The buccolingual thicknesses of the drilled region in the lower and upper jaws were measured with calipers, yielding 8.2mm for the upper jaw and 4.1mm for the lower jaw. Screws used in the upper jaws were shortened by about 3mm because it was noticed that the 6mm length screws would enter the nasal cavity in the upper jaws.

Surgical Procedure

The rabbits were anaesthetized with 8mg/kg intramuscular xylazine (Xylazin Bio® 2%,

Bioveta, Czechia) and 50mg/kg ketamine (Ketasol® 10%, Richter Pharma Ag, Austria). The perioral regions were shaved. Preoperative weights were determined with precision scales. The oral cavity and perioral region were disinfected with polyvinylpyrrolidone-iodine (Batticon® 10%, Adeka, Türkiye). For local anesthesia of each half of the jaws, 0.5ml articaine containing 1:200000 epinephrine (Ultracain® DS, Sanofi-Aventis, Türkiye) was administered.

The upper jaw was incised vertically in the distal part of the incisor tooth and horizontally along the line following ruga palatina. The flap was elevated. The bone was drilled 1cm away from the incisor tooth with a 1mm diameter surgical bur marked at 3mm. The screw was placed, and the flap was sutured with three resorbable sutures (Pegelak® 3.0, Doğsan, Türkiye) (Figure 2a-2d).

Similarly, the lower jaw was incised vertically in the distal part of the incisor tooth and horizontally along the line following the lip fold. The flap was elevated, and the bone was drilled 1cm away from the incisor tooth with a 1mm diameter surgical bur marked at 6mm. The screw was placed, and the flap was sutured with three resorbable sutures (Pegelak® 3.0, Doğsan, Türkiye) (Figure 2e-2h).

In all, 40 dental implant cover screws were placed in 10 animals, one screw per each half of the jaws. On the lower jaws, 6mm screws were placed. All screws applied to the upper jaws were shortened to 3mm before the surgery. Untreated screws were placed in the control group, and coated screws were placed in the experimental group. Animals in the same group were operated in random order, and all surgical procedures were performed by the same surgeon.

Post-Operative Care

All animals were placed in separate metal cages after the surgical procedure under standard conditions (temperature 22±2°C; humidity 55±5%; light/dark cycle 12/12h) with water and food ad libitum. They were fed with a soft diet for three weeks because the surgical region was the mouth. Rabbits were observed frequently to monitor food intake and activity. They were given analgesics (0.3mg/kg Meloxicam, Maxicam®, Sanovel, Türkiye) and antibiotics (50mg/kg cefazolin sodium, Cezol® 1gr I.M./I.V., Deva, Türkiye) intramuscularly twice a day for four days after the surgery.

Sacrification and Other Applications

To determine the most important result of the experiment, dental LED curing light (Woodpacker® LED.B, Guilin Woodpacker Medical Instrument Co. Ltd., China) was applied to all regions where the screws were placed just after the sacrification. The light was put in contact with the oral mucosas, and the light intensity was adjusted to the maximum and held for 20 seconds per region. The appearance of the screws under the mucosas was evaluated by two independent observers in

the operating room when the animals were alive. The observers stayed 30cm away from the lighted region, and the room was illuminated with daylight.

All animals' last weights were evaluated with precision scales. The oral mucosa that was in contact with the screws was excised approximately 2x1cm in each half of the jaws. Abdomens were opened to remove livers and kidneys. Each kidney was divided into two. Livers were cut into samples with 1cm between them. Samples were taken by the same pathologist and placed in 10% buffered formaldehyde.

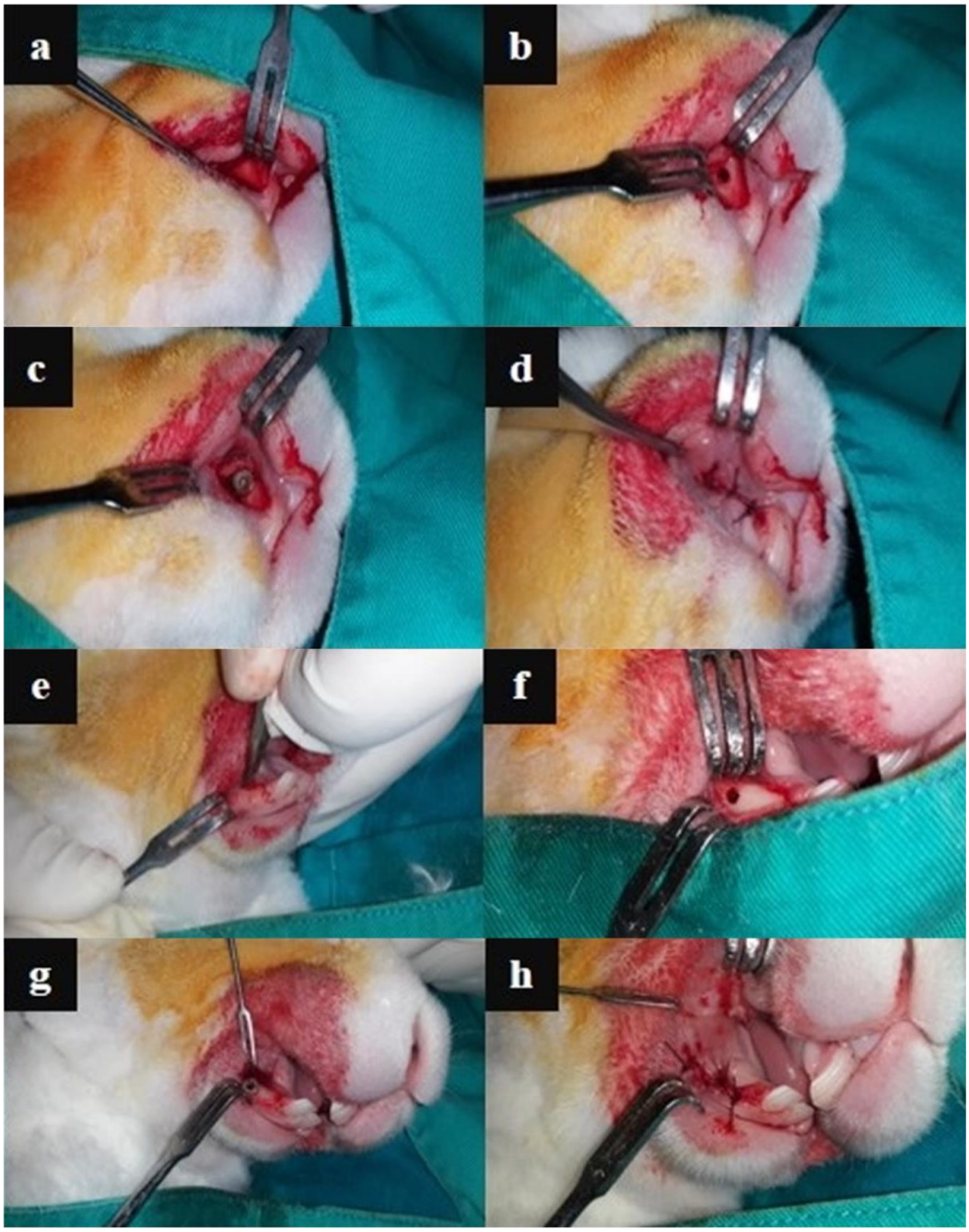

Figure 2. Intraoperative view after elevating of the flap, drilling of the bone, implanting of the screw and suturing of the surgery region. **a,b,c,d:** The upper jaw, **e,f,g,h:** The lower jaw.

Histopathological Preparations and Analysis

All tissues were fixed with 10% buffered formaldehyde for 48 hours. They were divided into small pieces for examination. Each piece was placed in a cassette and washed for 4 to 6 hours in running water to remove the formaldehyde completely. After that, they were placed in an automatic tissue processing device. Tissues were embedded in paraffin blocks. Paraffin blocks were cut at a 5-6µm thickness with microtome. Then all paraffin was removed in an oven. Tissues were stained with hematoxylin-eosin. Preparation of tissue samples was completed to examine them with a light microscope.

All prepared tissues were examined with a light microscope (Eclipse® E600, Nikon, Japan). The presence of an inflammatory reaction and the status of epithelialization were examined. The thickness of the oral mucosa epithelium was also measured using computer images obtained from the microscope. Differences between experimental and control groups were statistically evaluated.

Statistical Analysis

Preoperative and sacrification weights of all animals and epithelial thicknesses in each half of the jaws were compared statistically with a computer program (IBM SPSS Statistics® 20, Chicago, IL, USA). The normal distribution of all data was confirmed with the Shapiro-Wilk test. Independent samples were compared with independent samples T-test. The means of the preoperative and sacrification weights of the experimental and control groups were compared with paired samples T-test. Epithelial thicknesses were compared with paired samples T-test within the same group, and the intergroup epithelial thicknesses were compared with independent samples T-test. The significance level was chosen as 0.05 in all analyses.

RESULTS

Weights Comparison

To evaluate the homogeneity of the groups, preoperative weight values of the control and experimental groups were compared with the independent samples T-test (P=0.36). The distribution of the preoperative weights of groups was homogeneous (Table 1).

During the experiment, to see differences between the groups in terms of weights of the animals, the sacrification weights of the control group and of the experimental group were compared with the independent samples T-test (P=0.50). There was no statistical difference between them and both groups were affected by the process in the same way (Table 1).,

Table 1. Comparison of preoperative and sacrification weights (kg) between groups

		Minimum	Maximum	Mean	Median	Standard Deviation	p
Preoperative Weight	**Control (n=5)**	3.36	4.25	3.74	3.57	0.43	0.362
	Experimental (n=5)	2.25	4.22	3.30	3.74	0.90	
Sacrification Weight	**Control (n=5)**	2.10	4.03	3.22	3.10	0.75	0.501
	Experimental (n=5)	2.27	3.56	2.93	3.06	0.48	

To see the effect of the experiment on each animal, the differences between the preoperative and sacrification weights of all animals in the control and experimental groups were compared with the paired-samples T-test (P=0.30 for the control group; P=0.16 for the experimental group). It was concluded that the weights of the animals were not affected in either group by the experiment (Table 2). Europium and dysprosium doped strontium aluminate coating has no effect on weight loss or gains in rabbits.

Visibility of Screws Under Oral Mucosa Before and After the Light Application

Before the application of dental LED curing light to the oral mucosa where the screws were implanted, no findings were observed by the independent observers about the screws in both groups. After that, dental LED curing light was applied and the visibility of the screws under the oral mucosas was evaluated in both groups. It was determined that all screws implanted in the upper jaws became visible in the experimental group and they could be located under the mucosas by independent observers (Figure 3a). There was a green light in these regions that can be seen clearly in the room which was illuminated by daylight. However, all the screws implanted in the control group and on the lower jaws in the experimental group were not visible (Figure 3b). No findings regarding screws were found by both independent observers.

Histopathological Examination Finding

In the study, epithelization was normal and there was no inflammatory reaction in all oral mucosa regions in the experimental (Figure 4a-4e) and control (Figure 4f-4j) groups. In addition, the histological appearances of the livers and kidneys were normal, and no pathology was observed. A granulomatous infection was detected only in one oral mucosa region in the experimental group, which developed far from the contact region of the screw (the left lower jaw of the fifth rabbit in the experimental group). The reason was identified as food trauma and normal oral flora.

Table 2. Comparison of preoperative and sacrification weights (kg) in each groups

		Minimum	Maximum	Mean	Median	Standard Deviation	p
Control (n=5)	**Preoperative Weight**	3.36	4.25	3.74	3.57	0.43	0.296
	Sacrification Weight	2.10	4.03	3.22	3.10	0.75	
Experimental (n=5)	**Preoperative Weight**	2.25	4.22	3.30	3.74	0.90	0.156
	Sacrification Weight	2.27	3.56	2.93	3.06	0.48	

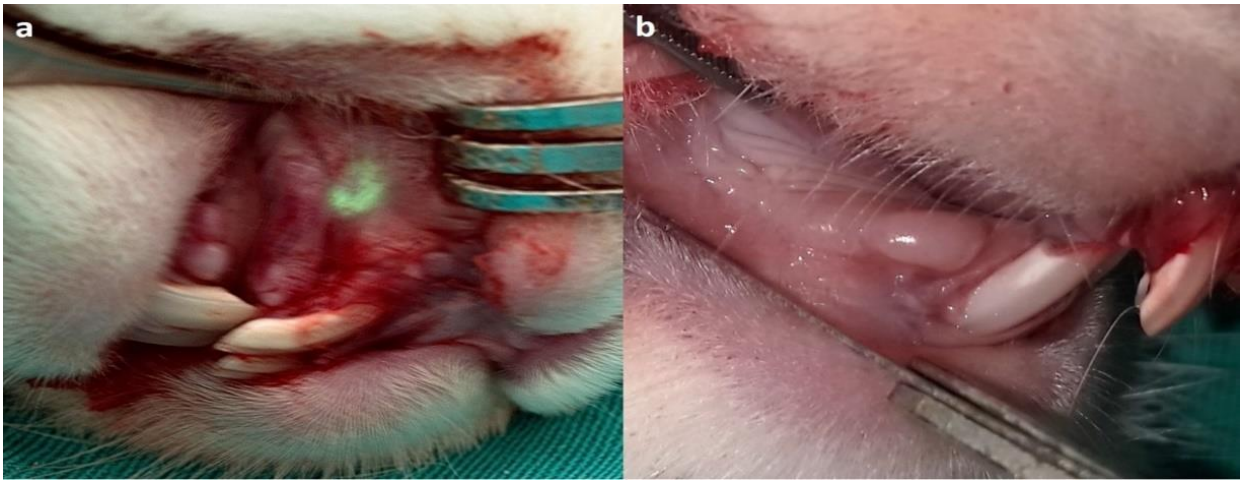

Figure 3. a: The screw's appearance after the light application in the upper jaw, **b:** The appearance in the lower jaw after the light application.

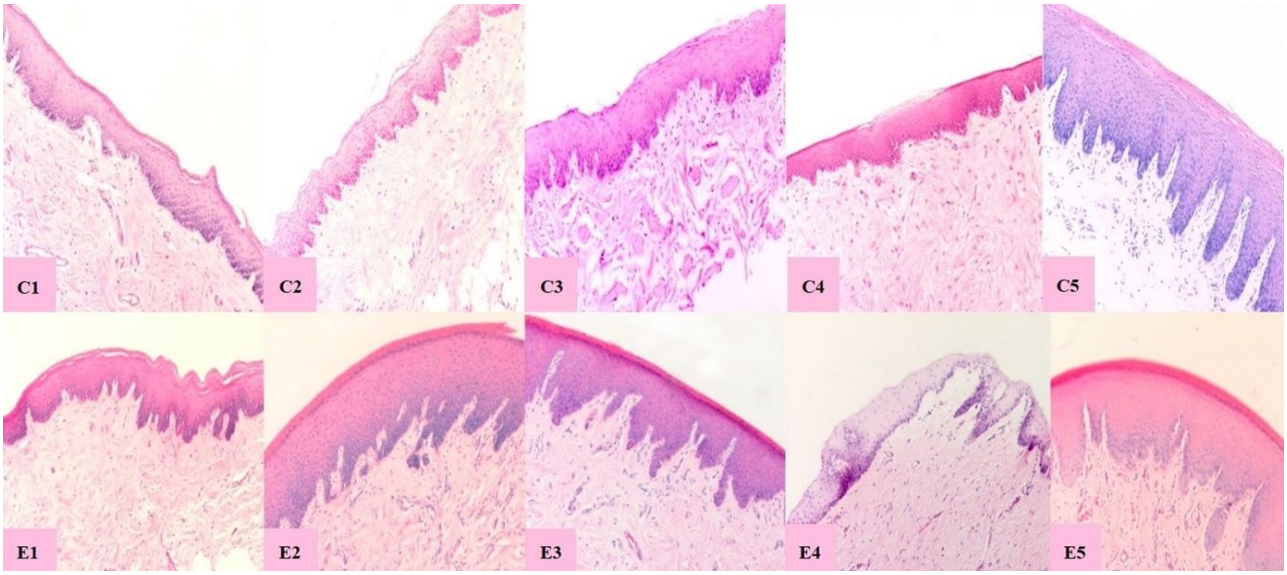

Figure 4. Histology of all animals' oral mucosa epithelium after hematoxylin-eosin staining (Magnification x10) (C= Control group, E= Experimental group).

Comparison of Epithelial Thickness

Epithelial thickness was measured at three different sites on each oral mucosa using computer images obtained from the microscope. The mean of these three measurements was determined and recorded as the epithelial thickness of these tissues.

Oral mucosa epithelial thicknesses in each half of the jaws between the control and experimental groups were compared with independent samples T-test (P=0.08 for right upper jaw; P=0.42 for left upper jaw; P=0.86 for left lower jaw; P=0.08 for right lower jaw) (Table 3). There was no significant difference between the oral mucosa epithelium thicknesses of each half of the jaws in the control and experimental groups. As a result, it was found that europium and dysprosium doped strontium aluminate coating did not affect epithelial thickness.

Table 3. Comparison of oral mucosal epithelial thickness (µm) of the control and experimental groups in each half of the jaws

		Minimum	Maximum	Mean	Median	Standard Deviation	p
Right Upper Jaws	**Control (n=5)**	132.630	224.560	173.236	173.120	38.250	0.081
	Experimental (n=5)	173.330	242.990	216.224	230.640	29.241	
Left Upper Jaws	**Control (n=5)**	114.430	398.840	273.508	304.390	110.256	0.417
	Experimental (n=5)	103.550	426.730	209.110	165.230	127.038	
Left Lower Jaws	**Control (n=5)**	182.270	463.190	346.312	354.640	102.866	0.856
	Experimental (n=5)	185.080	487.760	359.176	354.770	113.256	
Right Lower Jaws	**Control (n=5)**	259.890	426.410	322.390	323.140	65.967	0.078
	Experimental (n=5)	363.840	447.630	390.110	373.330	35.688	

DISCUSSION

The importance of dental implant surgery has been increasing with the developments of novel implant designs, surface properties and surgical techniques that offer many options for surgeons. The techniques are very important in oral implantology, as they are in all surgical sciences. In oral implantology, there are two techniques for implant placement, one- or two-stage surgery (8). Many studies have compared the two techniques. Both have advantages and disadvantages, but no consensus has been reached about which one is better (8-27).

Two-stage surgery is preferred more by practitioners. Dental radiograph is the most used method to determine the location of an implant under the oral mucosa after osseointegration. Scalpels are chosen mostly to use in the second surgery. Sometimes, dentists detect implant sites incorrectly. The incision is expanded to find the implant after such a failure, and over-incised regions are sutured. No infection usually develops, and antibiotics are not prescribed after this procedure. However, if an infection develops, dentists prescribe antibiotics. Incorrect or over-

incisions delay patients' prosthetic treatment stages (28).

Two-stage surgery has the disadvantage of requiring a second surgery, which takes more time and increases scar formation and treatment costs. Thick oral mucosa, dentists' lack of experience and multiple implant applications in edentulous cases make second surgery difficult. In some cases, the procedure can be performed with the elevating flap from a large region, as in the first stage (29). However, one of the important subjects in all surgical sciences is the minimal incision that provides optimum access to the surgical site. Over-incisions increase the risk of complications after surgery (30).

Flapless and conventional implant surgeries were compared, and less resorption in the neck region of the implant body with flapless surgery was reported (31). In some studies, flap elevation was found to induce bone resorption (32,33). These studies prove that excessive or wrong incisions and elevating the flap have negative consequences when replacing the cover screws in the second stage of the two-stage surgery.

All this information made it necessary to develop a new, minimally invasive method in two-stage implant surgery that can determine the exact location of the implant under the oral mucosa. For this purpose, a study using phosphorescent pigment coated implant cover screws and dental LED curing light has been designed.

In this study, rabbits were preferred because of the jaw structures suitable for screw placement. It was planned to place screws in each half of the jaws to obtain maximum data from a minimum number of animals. Europium and dysprosium doped strontium aluminate coated implant cover screws were surgically implanted in the experimental group, while untreated implant cover screws were used in the control group. At the end of six weeks that is necessary for implant osseointegration in rabbits (34), the visibility and biocompatibility of the screws under the oral mucosa were investigated.

Before the light application, there was no sign about screws in all rabbits. After that, the light was applied to both groups to determine whether the screws under the mucosa are visible. All screws were clearly observed in the upper jaws in the experimental group. However, all the other screws were not seen. This is the most important result of the study.

Oral mucosa regions in contact with the screws were excised to investigate the local biocompatibility of the europium and dysprosium doped strontium aluminate coating. To evaluate general biocompatibility, livers and kidneys were removed based on other toxicity and biocompatibility studies in the literature (35-38). All tissues were stained with hematoxylin-eosin and examined with a light microscope. Histopathologically, no inflammatory reaction was observed in any

tissues. Epithelialization of the oral mucosas, epithelial cells, and underlying connective tissues were normal. Livers and kidneys were healthy. Thus, it was determined that europium and dysprosium doped strontium aluminate is biocompatible for these tissues.

Increasing epithelial thickness is one of the most important findings in oral malignity (39). Therefore, the epithelial thickness of each oral mucosa was measured. The epithelial thicknesses of the control and experimental groups on each half of the jaws were compared. At the end of the comparison, there was no statistically significant difference between the epithelial thicknesses of the groups. Thus, it was proved that europium and dysprosium doped strontium aluminate has no effect on epithelial thickness.

The preoperative and sacrification weights of each animal were measured with a precision scale. There was no statistically significant difference between the two groups in terms of preoperative weight. This shows that two randomly separated groups were homogeneously distributed in terms of their weights. Similarly, there was no statistically significant difference between the two groups in terms of sacrification weight. This indicates that animals were equally affected by the process. Finally, preoperative and sacrification weights of each animal were compared, and no statistically significant difference was found. Thus, it was proved that normal screws and europium and dysprosium doped strontium aluminate coated screws have no weight effects on animals.

Europium and dysprosium doped strontium aluminate is the easiest to acquire, most used and researched, long-term and powerful light-emitting phosphorescent pigment. The strongest light is produced by europium and dysprosium doped strontium aluminate among all long persistent phosphorescent pigments (4). HeLa cell cultures were treated with europium and dysprosium doped strontium aluminate at four different concentrations (0.001, 0.01, 0.1, 1µg/µL), and the study showed that europium and dysprosium doped strontium aluminate is not cytotoxic at any of these concentrations (2). In safety data sheets published by manufacturers, oral and dermal acute toxicity LD50 doses for rats are reported to be >2000mg/kg and the chromosomal aberration test about reproductivity is evaluated to be negative (3). For these reasons, europium and dysprosium doped strontium aluminate was used in this study.

Different methods have been found in the literature to determine the exact location of implants. An ultrasonic device was developed to locate submerged implants (40). It can detect implants that are under the oral mucosa that is up to 5mm thick in pigs. But the system is not reliable in implants with more than 5mm of mucosal thickness. Additionally, the need for a special device for detecting is disadvantageous.

In a previous study, paint was injected into the oral mucosa at the end of the first stage to detect the exact location of the implant (29). In 87.5% of the cases, implants were clearly found in the second stage of the surgery. The biopsies showed that the paint is biocompatible for the tissues and no foreign body reaction developed. This is a very useful and economical method. However, the excessive injection can be done by mistake in aesthetic regions. In addition, even though the paint is biocompatible for the body, a foreign material will remain permanently.

Another method for locating implants under the oral mucosa is the use of electronic devices (29). A sensor is moved inside the mouth, and it gives an audible, light warning when it detects an implant. Then the site is marked, and the mucosa on the implant is excised or incised. There are some disadvantages such as the expensive cost of the device or the incorrect results that may be gained due to the low battery or deformation of the sensor.

Our study is considered an alternative method that uses dental LED curing light and coated cover screws with phosphorescent pigment. This technique does not add an additional process to the conventional two-stage surgery. There is no need for a new device, and the production cost of the screws is very low. Also, the location of the screws can be found exactly, quickly and without radiographs.

Mainly, the visibility of phosphorescent coated screws under live tissues after a light application was evaluated in this study. In addition, their biocompatibility was investigated. For these reasons, some other analyzes have been limited. The most important limitation of this study is that it is not possible to determine what the maximum thickness of the tissue should be to see the coated screws. They could be measured with a periodontal probe or a needle after implantation or before sacrification. But due to the risk of tissue injury during the measurement, it was not performed in both times. Nevertheless, it was seen that all soft tissues on the lower jaws are thicker than on upper jaws. Very thick folds on the lower jaws were observed just above all screws. This could be why the screws could not be detected in the lower jaws in the experimental group.

The other limitation is exactly unknown general biocompatibility. Liver and kidney examinations and previous studies give an idea, but this is not precise. Further studies are needed to reveal these situations.

CONCLUSION

In this study which examined the effects of europium and dysprosium doped strontium aluminate coated titanium materials on rabbits, a different method was developed for the second stage of two-stage dental implant surgery even though there are some limitations. The coated screws were detected under the oral mucosa. In addition, this material was found to

be biocompatible for examined tissues. In the future, maybe this method can also be applied in humans after necessary steps are fulfilled, and a minimally invasive procedure will arise that is different from the conventional methods in the two-stage implant surgery.

Ethics Committee Approval: The study protocol was approved by the Ondokuz Mayıs University Animal Care and Ethics Committee with project number 2017/07.

Author Contributions; Concept: EY, EB, NK, TG, Design: EY, Data Collection and Processing: EY, Analysis and Interpretation: EY, EB, Writing: EY, EB,

Conflict of Interest: The authors have declared that no conflicts of interest exist.

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