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Evaluation of the Effect of Various Therapeutic Agents on Osseointegration of Dental Implants: Complications in A Rabbit Tibia Model

Çeşitli Terapötik Ajanların Dental Implantların Osseointegrasyonu Üzerine Olan Etkisinin Incelenmesi: Tavşan Tibia Modelinde Görülen Komplikasyonlar

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ABSTRACT

Objective: The aim of our study is to evaluate the complications that occurred on rabbit tibia bones after implant placement.

Material and Method: In our study, 10 adult male white healthy New Zealand rabbits (average weight: 3,000 to 3,500 g) were used. Different numbers of dental implants were placed in the experimental animals. Complications in rabbit tibias were observed and radiological results were reported.

Results: Of the 10 rabbits included in the postoperative study, one (10%) died immediately after the operation, eight (80%) within the first 24 hours, and the last one (10%) died within the next 48 hours of the operation.

Conclusion: Based on the complications experienced during this study and the data we obtained, we conclude that the rabbit tibia is not suitable for such studies. More comprehensive and multi-center studies are needed to draw more firm conclusions on this subject.

Keywords: *Dental implant, Experimental animals, Rabbit, Complications*

ÖZET

Giriş: Bu **ç**alışmanın amacı, yerleştirilen implantlar sonrası tavşan tibia kemikleri üzerinde meydana gelen komplikasyonları değerlendirmektir.

Materyal ve Metot: Bu **ç**alışmada sağlıklı (ortalama ağırlığı 3000–3500 g), 10 adet yetişkin erkek, beyaz Yeni Zelanda tavşanı kullanılmıştır. Deney hayvanlarına farklı sayılarda (1-3) dental implant yerleştirilmiştir. Tavşan tibialarındaki komplikasyonlar gözlenmiştir ve radyolojik sonuçları bildirilmiştir.

Bulgular: Operasyon sonrası çalışmaya dahil edilen 10 tavşandan biri (%10) operasyondan hemen sonra, sekizi (%80) operasyon sonrası ilk 24 saat içerisinde ve kalan biri (%10) sonraki 48 saat içerisinde kaybedilmiştir.

Sonuç: Çalışma süresince yaşanan komplikasyonlar ve elde edilen verilere göre bu tür çalışmalar için tavşan tibiasının uygun olmadığı sonucuna varılmıştır. Kesin sonuçlar için daha kapsamlı ve çok merkezli çalışmalara gereksinim vardır.

Anahtar kelimeler: *Dental implant, Deney hayvanları, Tavşan, Komplikasyon*

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INTRODUCTION

Tooth loss has been a physically and psychologically traumatic and devastating experience for humans throughout the history. To overcome this problem, the application of dental implants, which have been frequently used in recent years, were first described in the 1960s by Branemark et al. with the discovery of osseointegration (Branemark, 1977). However, frequent use of dental implants in dental care has increased the complication rate (Pjetursson et al., 2018). Some of these complications can be successfully treated, while some require high cost and involve difficult treatment process for both dentists and patients (Appukuttan, 2016).

The implant failure rate varies between 5 and 7% in the literature (Francetti et al.,2019). Failure to achieve osseointegration resulting in implant loss is one of these undesirable situations (Esposito et al., 1998). The term osseointegration is defined as the direct osseous connection between the living bone tissue and the implant, which is formed without fibrous connective tissue and seen at the level of the light microscope; i.e., a structural and functional union (Branemark, 1977). Therefore, the prerequisite for the success of dental implants is the formation of a strong osseointegration between the implant and bone after placement (Marco et al., 2005). However, there are several factors which affect the osseointegration and, thus, the healing process directly or indirectly such as the quality of the bone in the surgical site, vertical bone height, bone width, nutrition, systemic condition of the patient, oral hygiene, and surface properties of the implant (Branemark, 1977).

Previous studies aiming at increasing the implant success rates have used various animal models such as rat, mouse, sheep, pig, and rabbit. To date, rabbits are the most commonly used animals in studies with a rate of about 35% (Neyt et al.,1998). Experimental studies in rabbit models have primarily used tibial and femoral condyles to evaluate bone-implant surface. Sennerby reported that the tibial metaphyseal region could be used to stimulate the mandible and the more cancellous femoral epiphysis region could be used to stimulate the maxilla, as it has a more compact structure (Sennerby et al., 1992). The cortical bone thickness in rabbit tibial diaphysis and metaphysis is about 1.2 to 1.5 mm. The tibial bone length in an adult rabbit is about 11 cm (Gilsanz et al., 1998). In the light of these data, the maximum number of implants which can be applied in the long bones of rabbits is estimated as six in accordance with the international standards (Gilsanz et al., 1998).

In this experimental study, we aimed to evaluate the dental implants procedure-related complications in a rabbit tibia model.

MATERIAL and METHOD

Study design and study subjects

This study was approved by the Animal Experiments Ethics Committee of XXX (No: 2019/01, Date: 31/01/2019). And it was designed according to the NIH Guidelines for the Care and Use of Laboratory Animals. A total of 10 adult male white New Zealand rabbits with an average weight of 3,000 to 3,500 g which were determined to be healthy by veterinary control were used. The rabbits were placed in standard experimental cages and standard laboratory diet was applied at 22 to 24°C, 55 to 70 humidity, 1 atm, and 12-hour light and dark cycle. Two weeks before the experiment, the rabbits were placed in their cages to adapt to the laboratory setting and their health status was observed on a regular basis.

The study was designed as implant placement both tibia of rabbits.

Surgical procedure

All surgical procedures were carried out under sterile surgical conditions following the rules of asepsis, antisepsis, and sterilization. General anesthesia was performed using intramuscular xylazine (Rompun®; Bayer Pharmaceuticals, Istanbul, Turkey) and ketamine hydrochloride (Ketalar®; Eczacibasi Warner Lambert Pharmaceuticals, Istanbul, Turkey). Following anesthesia, the tibia bones of the rabbits were shaved and disinfected with povidone-iodine. After the surgical site preparation, the full-thickness flap through incision and dissection was lifted and the bone tissue was exposed. A 3,5 mm wide and 10-mm deep sockets were created on the right and left tibia using implant burs (NucleOSS Implant, Izmir, Turkey) with a physiodispenser at 800 rpm under water cooling. Implants and socked were irrigated with therapeutic agents (Rifampicin, Calcium D and Saline). Dental implants were, then, placed in the sockets prepared with a torque of 25 to 35 using a micromotor (Figure 1).

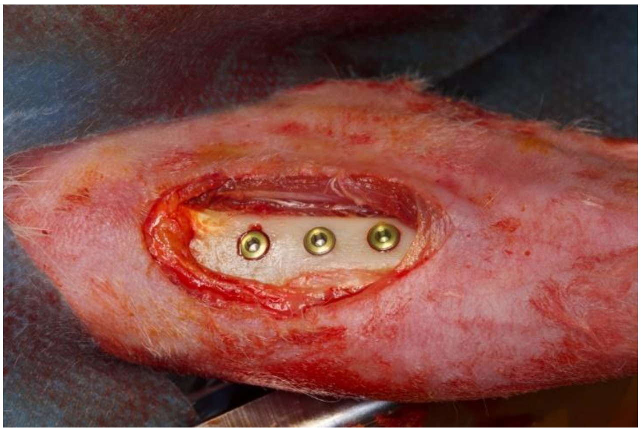

Figure 1. Dental implants placed in created sockets.

The surgical site was washed with saline and bleeding control was performed. The subcutaneous tissues were sutured primarily with absorbable sutures (Serapid®;5-0 Serag-Wiessner GmbH & Co. KG, Naila, Germany) and the skin with monofilament absorbable sutures (Serafast®;5-0 Serag-Wiessner GmbH & Co. KG, Naila, Germany) (Figure 2).

In order to avoid bone fracture, three implants were placed in each tibia of two rabbits, then two implants in each tibia of two rabbits, and finally one implant in each tibia of the remaining six rabbits.

Figure 2. Suturing the surgical field

RFA application

Primary stability was evaluated using the Osstell™ Mentor (Integration Diagnostics AB, Göteborg, Sweden) device. The SmartPegs™ (Integration Diagnostics AB, Göteborg, Sweden) were placed on the implants and tightened by applying finger pressure in accordance with the manufacturer's instructions. Two measurements were performed in the mesio-distal and bucco-lingual directions bringing the tip of the Osstell™ Mentor device close to the SmartPegs™ at an angle of 90° until 2 to 3 mm away (Figure 3). The implant stability quotient (ISQ) score was recorded for each implant by taking the arithmetic average of all measurements. The initial ISQ measurements were made, while the measurements at Week 4 could not be made, as the study was prematurely terminated as a result of complications after the surgical procedure.

Figure 3. The ISQ measurements of dental implants.

Periotest® application

The Periotest® device is used to analyze electronical signals by an accelerometer and consists of a handpiece mounted to a percussion rod and a metal tip. The stability values of the implants were recorded using a Periotest® (Medizintechnik Gulden e.K., Modautal, Germany) device in our study (Figure 4). However, the study was prematurely terminated, only the initial measurements could be obtained and the measurements at Week 4 could not be recorded.

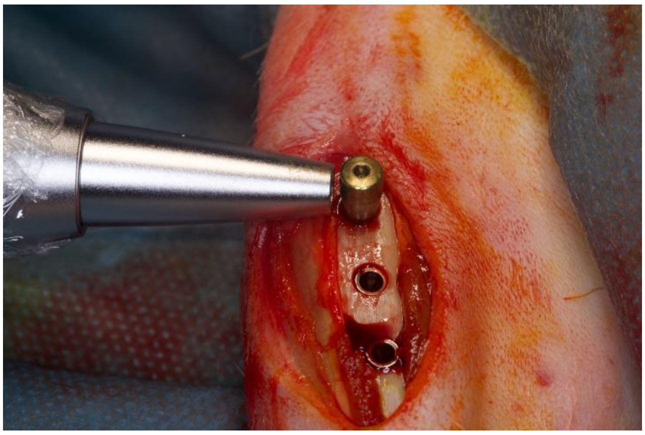

Figure 4. Stability measurements using a Periotest® device

Histopathological examination

At the beginning of the study, we planned to sacrifice the subjects at the end of the fourth week and obtain 10-mm biopsy specimens, including the bone and implant using a 5-mm a trephine bur in each implant site. We also planned to store these samples in formaldehyde at room temperature until analysis at the Department of Periodontology and perform histopathological analysis. However, as the study was prematurely terminated, histopathological analysis could not be performed.

Statistical analysis

Descriptive statistics for categorical variables in this study were expressed as numbers (n) and percentage (%). SPSS (IBM SPSS for Windows, ver.26) statistical package program was used for the calculations.

RESULTS

Fractures were observed in the cortical layers of the rabbit tibia, although we made a great effort to prevent fractures during implant placement. As a result, the number of implants placed for each tibia was reduced.

During the study, the animals had torsion in the surgical field with an abnormal mobility in the bone tissue. Of the 10 rabbits included in the postoperative study, one (10%) died immediately after the operation, eight (80%) within the first 24 hours, and the remaining one (10%) died within the next 48 hours of the operation. As a result of these complications, the study was prematurely terminated at the discretion of the principal investigator and with the approval of the Ethics Committee. The rabbits were sacrified immediately after their deaths.

The operated tibiae of the rabbits were removed after sacrification and direct radiographs of the tissue specimens were obtained using digital radiography equipment (flat cassette, 150 KW). The clinical and radiographic examinations in the postoperative period revealed fractures in the tibias of the subjects and the presence of edema in the adjacent tissues on radiographs (Figures 5 and 6). Subsequent analyses of the study were unable to be carried out, as the study was terminated due to complications and unexpected deaths.

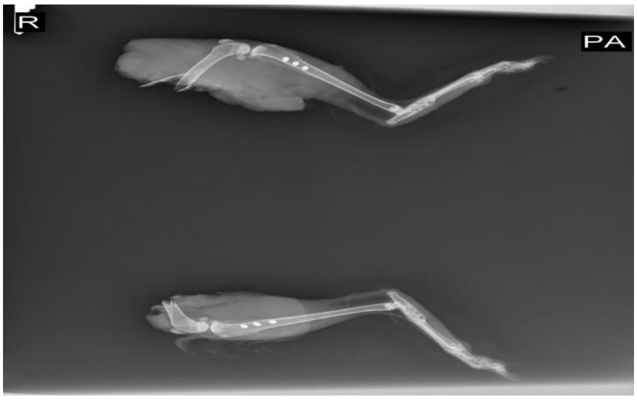

Figure 5. Radiographs of the sacrified tibia and adjacent tissues in axial, coronal, and sagittal plane

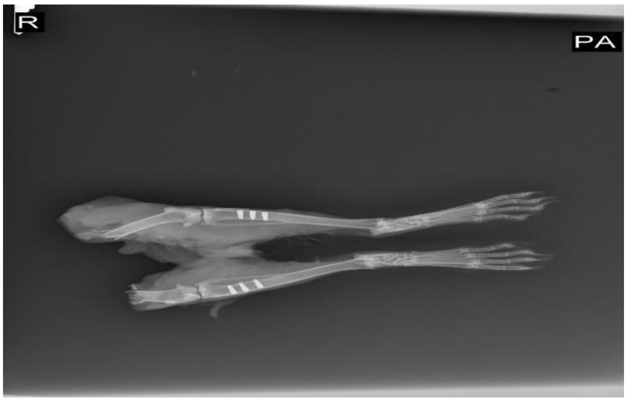

Figure 6. Radiographs of the sacrified tibia and adjacent tissues in axial, coronal, and sagittal plane

DISCUSSION

Currently, dental implant treatment and its success mainly depend on osseointegration in the practice of dentistry (Saykaras et al., 2000). For an ideal osseointegration, the response of the biological tissue around the implant is critical. This response is closely linked to the surface properties of the implant including surface modifications, chemical content, coating, and sterilization processes and several studies have been conducted on this subject in the literature (Shi et al., 2016). There are many animal studies in rats, mice, sheep, pigs, and rabbits investigating the impact of accelerating the osseointegration between the bone and implant by changing the surface properties and designs of the implants (Lang et al., 2004).

The rabbit model is widely used in experimental studies on the musculoskeletal system, although their size and shape vary (Lang et al., 2004). Previous studies used tibial and femoral condyles of rabbits to evaluate bone-implant surface (Del Fabbro et al., 2006). In a study, Sennerby et al. (1992) reported that the tibial metaphyseal region could be used to stimulate the mandible and the more cancellous femoral epiphysis region could be used to stimulate the maxilla, as it has a more compact structure. In the light of literature data, we used New Zealand rabbits in our study. However, we prematurely terminated this study due to unexpected complications at the discretion of the principle investigator and with the approval of the Ethics Committee. Although there are many reasons for these complications, there is not enough information on this subject in the literature. Among the possible reasons, the lack of appropriate animal selection can be considered. However, the animals used in this study were supplied from two centers in equal numbers. Inappropriate care and feeding of rabbits may also cause such complications; however, the experimental animals used in this study underwent veterinary control before the study and approval was obtained from the physician. All animals were also fed properly and each animal was placed in separate cages during the preparation phase and after the study. Taken together, complications were not related to the age, weight, or care of the animals.

There are differences and similarities between the rabbit and human bone. One of the main differences is that the rate of bone turnover is remarkably higher in rabbits (Hoffmann et al., 2012). Unlike the literature, recent studies have shown that rabbit bones are the least similar to human bone tissue among various species in terms of micro- and macrostructure of the bone (Martiniakova et al., 2005; Pearce et al., 2007). The most suitable species for use in implant research are sheep, goat, and dog models (Pearce et al., 2007). In the light of these data, the use of rabbits in implant research is questionable. Unexpected complications and results in our study support that the preference of the rabbit tibia model for implant research may lead to undesirable outcomes.

The cortical bone thickness in rabbit tibial diaphysis and metaphysis is about 1.2 to 1.5 mm. The tibial bone length in an adult rabbit is about 11 cm (Gilsanz et al., 1998). In the light of these data, the maximum number of implants which can be applied in the long bones of rabbits is estimated as six in accordance with the international standards (Gilsanz et al., 1998). In the current study, we planned to place implants in both tibia. However, we gradually reduced this number due to unexpected complications. Although the anatomical and morphological structure of the rabbit tibia have been considered suitable for such studies, we observed fractures in the cortical layer during implant placement in our study. We also found that these fractures increased proportionally to the number of implants placed.

Although previous studies have suggested that rabbit tibia is morphologically suitable, we experienced problems related to implant stability during implant placement. We fully followed the surgical drilling protocol during the preparation of the implant sockets; however, the stability problem may have resulted from morphological differences. It has been well established that human alveolar bone morphology can be divided into four categories as D1, D2, D3 and D4, while the morphology of the lower and upper jaws is mostly at the D2 and D3 levels (Misch, 2007). The stability problem in our study can be presumably attributed to the fact that the cortical layer of the rabbit tibia is at the D1 level and the cancellous layers at the D4 level.

In the present study, we used neck burs and tapers during dental implant surgery to reduce the stress on the implant neck as recommended in the literature, we observed problems related to implant stability, indicating that the morphology of the rabbit tibia needs a different protocol. Therefore, this should be considered in implant studies where the rabbit tibia model is used. It is also possible to avoid the use of such burs to prevent possible loss of stability.

Previous studies have shown that fractures in the skeletal tissue are the most common cause of death in rabbits (Mapara et al., 2012). Similarly, in our study, we observed fractures in the tibia of the subjects and we gradually reduced the number of implants. We believe that tibia fractures and torsion on all rabbits are related to morphological unsuitability of the rabbit tibia for implant research, rather than the protocol followed.

In most studies, bone fractures have not been described in rabbits. However, a study using rabbits as an animal model for experimental research showed that the main complication was fracture development during and after the operation, similar to our study (Mapara et al., 2012). In this study, the rabbits could not maintain a postoperative bandage of cast and removed it biting, which raises questions about the use of the rabbit model for experimental studies on bone tissue.

Death of the rabbit within 24 to 48 hours after fracture is common due to fat embolism as evidenced by the post-mortem studies (Mapara et al., 2012). Similarly, in our study, all death events occurred within 24 to 48 hours after the fracture. Although this finding suggests that analgesia protocol may be inappropriate, we used the protocol as described in the literature and carried out all procedures under the supervision of a veterinarian.

According to the literature, an implant size of 2 mm in diameter and 6 mm in length is recommended for rabbit bone (Mapara et al., 2012). However, we used larger and longer implants in our study, which may have resulted in unexpected complications. On the other hand, some studies used implants larger than the implant size and diameter used in our study (Scarano et al., 2018; Sanchez-Perez et al., 2020). This makes the use of rabbit models in such experimental studies questionable.

Conclusion

In conclusion, in the present study, we attempted to investigate the effect of various therapeutic agents on osseointegration of dental implants in a rabbit model. For this purpose, we carried out animal selection in accordance with the literature and per protocol. Despite all efforts, the study was prematurely terminated due to unexpected complications. Based on these results, we believe that the rabbit tibia is not a suitable model for implant research. Nevertheless, further comprehensive, multi-center, large-scale studies are warranted to draw more reliable conclusions on this subject.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical Approval: This study was approved by the Van Yuzuncu Yil University, Faculty of Dentistry, Animal Experiments Ethics Committee with the Approval No: 2019/01 and Date: 31/01/2019.

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