Research on the model of mandibular alveolar bone defect in rabbits

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Abstract

Objectives: The purpose of this study was to create an animal model of a mandibular alveolar bone defect without compromising the animal's well-being. **Materials and methods**: A total of 24 New Zealand white rabbits underwent surgery to create mandibular alveolar bone defects. The animals were sacrificed at 2, 4, 6, 8, 10, and 12 weeks post-surgery. To assess bone regeneration at the surgical site, radiography, dental cone-beam computed tomography (CT), and histological examination using Hematoxylin and Eosin staining were performed on the skull. **Results**: A straightforward and easily executable method was devised to create the rabbit mandibular alveolar defect model. After 10 weeks, complete soft tissue and bone regeneration were observed. X-ray and cone-beam CT evaluations demonstrated a progressive increase in bone density from weeks 2 to 12. Histological examination revealed that the alveolar bone structure was formed incrementally at the surgical site. The bone and connective tissue had filled the defect after 8 weeks. **Conclusion**: The creation of a model of mandibular alveolar bone defects in rabbits is a straightforward process that can be used to assess the regeneration of alveolar bone at the defect site. This animal model can serve as the foundation for tests to evaluate the capacity of biomaterials to regenerate the alveolar bone.

Keywords*: mandibular alveolar bone, Alveolar bone defects, animal models, bone regenerative medicine, tissue engineering.*

1. INTRODUCTION

The alveolar bone, which is a component of the upper and lower jawbones, encircles and supports the teeth. In certain instances, the alveolar bone may be damaged by trauma, jaw tumors and cysts, infection, or tooth loss [1]. Furthermore, periodontitis is another factor that contributes to bone loss and alveolar bone defect development [2]. Alterations in the shape and structure of the alveolar bone not only affect the ability to chew, but can also lead to aesthetic, comfort, and confidence issues for patients, necessitating re-treatment. Therefore, the restoration of alveolar bone defects in patients is essential. In the context of replacing missing teeth, reconstructing the bone morphology in the jaw ridge is crucial for ensuring the stability of the restoration and fulfilling the aesthetic and functional requirements of the patient [3].

Alveolar bone defects are a prevalent issue in Maxillofacial Surgery due to a variety of reasons [4]. These defects can heal slowly or not at all because of factors such as large size, unstable physiological characteristics, subpar surgical techniques, or external influences such as metabolism, hormones, nutrition, and stress [5]. Therefore, reconstructing alveolar bone defects to restore both function and aesthetics is a major challenge for maxillofacial surgeons. Addressing alveolar bone defects typically involves surgical intervention and the use of bone grafting techniques and other healing aids [6]. Bone grafting aims to stimulate or facilitate new bone growth to fill defect [7].

Researchers have investigated various materials, including autologous bone, tissue-engineered materials, stem cells, and growth factors, to address bone defects [8]. Autologous bone derived from the patient's own body is considered the optimal choice because of its ease of use, low cost, and ability to perform bone graft surgery simultaneously. However, the removal of autologous bone can result in significant consequences for the patient, such as prolonged recovery time, infection, bleeding, and nerve damage [9]. To overcome these limitations, artificial bone powders with desirable biological properties such as Hydroxyapatite and Beta-Tricalcium Phosphate have been developed. Biphasic Calcium Phosphate, a mixture of Hydroxyapatite and Beta-Tricalcium Phosphate, have been

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developed. Biphasic Calcium Phosphate, a mixture of Hydroxyapatite and Beta-Tricalcium Phosphate, possesses higher compressibility, radiopacity, and bone formation capabilities than either component alone. Consequently, Biphasic Calcium Phosphate has been employed in dentistry and orthopedics to address various bone defects, including those caused by periodontal disease, tumors, and large facial defects [10].

The use of biological models is crucial for conducting material testing studies and evaluating the effectiveness of bone formation and the healing ability of bone grafting techniques before clinical application. Animal models with alveolar bone defects are particularly suitable for research aimed at assessing the ability to regenerate alveolar bone, as demonstrated in studies by Kamal et al. (2017) on a rabbit alveolar bone defect cleft model [11], Koh et al. (2018) on a mouse alveolar bone defect model [12], and Cakir's research group (2019) on a synthetic bone powder mixed with platelet-rich fibrin material in sheep [13]. Similarly, Seek et al. (2019) investigated the effects of platelet-derived materials (platelet-rich fibrin) in treating alveolar bone defects in dogs [14].

2. MATERIAL AND METHODS

Animals and housing

The research was performed on 24 male New Zealand white rabbits, purebred and weighing 2.5 ± 0.2 kg, aged 8 - 10 weeks. All participating rabbits were housed in a controlled laboratory environment at a room temperature of 25°C and a humidity of 56%. A 12-hour light/dark cycle was maintained. The rabbits had unrestricted access to standard laboratory food pellets and water. Rabbits with postsurgical complications, such as wound dehiscence or signs of infection, or those that died before the conclusion of the study, were excluded from the study.

Alveolar bone defect model creation surgery

The process of creating a model of mandibular alveolar bone defect in rabbits was inspired by the method outlined by Shad et al. in 2016 [15]. All the rabbits were housed in individual cages and received equal care, including food and water, throughout the study. Before surgery, the rabbits were anesthetized with xylazine HCl (5 mg/kg) and ketamine HCl (35 mg/kg) administered intravenously. The rabbit's neck and left side of the face were shaved, and the operating table was disinfected with 70% ethanol. The rabbit was then placed in a supine position on the operating table with its legs secured to the four corners using soft straps, and a folded towel was placed under the operated side of the animal's head to elevate the area. Anesthetic penetration was assessed by gently tapping the feet.

The surgical area was disinfected with a 5% povidone-iodine solution, and local anesthesia was administered using 2% lidocaine. Create a 20 mm long incision on the lower border of the left mandible, passing through the subcutaneous tissue and lower border of the mandibular body. A periosteal dissection was performed along the lower border of the mandibular body to expose the lateral mandibular surface. An 8 mm trephine bone cutter was then placed in the molar area at least 2 mm away from the upper and lower edges of the mandible and drilled through the outer bone, tooth roots, spongy bone, and inner bone of the lower jaw. Physiological saline was sprayed during the drilling process, and bone fragments were removed with a root picker to create a cylindrical bone defect 8 mm in diameter. The defect area was cleaned with physiological saline and then closed in three layers: the muscle, fascia, and skin.

Evaluation of bone regeneration by X-ray analysis

Bone healing was observed using X-rays at three different time points: two weeks, four weeks, and eight weeks post-surgery. The radiograph of the tooth root was positioned parallel to the alveolar bone defect on the surgical side of the mandible and captured with a current of 65 kvp and 7.5 mA for 0.16 seconds. The images were analyzed using EZDent biomedical software [16].

Assess bone density in the defect area using cone beam CT

To assess the process of new bone formation at the defect site, cone beam CT was conducted at 2, 4, 6, 8, 10, and 12 weeks after surgery. Before scanning, the bone sample was immersed in gauze soaked in 70% ethanol and then wrapped with parafilm to create a barrier that prevented moisture from escaping. It was crucial that the specimens remain moist but do not drip during the scanning process, which takes place under constant conditions [15].

On a 15.6 inch flat screen computer with a resolution of 1920 × 1080 pixels and Rainbow 3D Viewer 1.1.0 software, CT images were observed. The location of the defect was recorded, and the origin of the coordinate axis was moved to the center of the defect in the horizontal plane (axial). A vertical line was cut along the outside-inside

direction by dividing the defect into two equal parts. In the Coronal plane, the vertical cutting line was adjusted along the mandibular axis and passed through the center of the defect.

Histological evaluation of bone formation and connective tissue

A tissue sample measuring 1.5 × 3 cm in size, which included the area of the alveolar bone gap, was fixed in a 10% paraformaldehyde solution for 24 hours. Following this, the sample was demineralized, embedded in paraffin wax, and sliced perpendicular to the depth of the defect at a thickness of 5 µm in the center of the alveolar bone defect, resulting in three specimens. These specimens were stained using Hematoxylin and Eosin. The histological structures were visualized using a light microscope at three different magnifications: 40x, 100x, and 400x. The formation of connective tissue and new bone within the defect area should be examined using histologically stained specimens. The alveolar bone cell nucleic acid components stain dark blue, while the connective tissue protein components stain red to pink [17].

3. RESULTS

3.1. The capacity for regenerating alveolar bone defects was assessed using radiography

The complete structure of the mandible, including the teeth and the surrounding alveolar bone, is shown in Figure 1. a. After creating a defect and removing a certain amount of alveolar bone, as shown in Fig. 1. b shows the absence of a large amount of connective tissue and bone, leaving a circular defect. At 2 weeks, the defect was primarily filled with connective tissue and the surrounding bone defect boundary was clear (Figure 1.c). At 4 weeks, there was limited bone regeneration at the edge of the defect, and new bone was formed in the connective tissue (Figure 1. d). At 6 and 8 weeks, new bone formation increased, but bone regeneration did not fill the defect (Figure 1e, f). At 10 and 12 weeks after surgery, bone formation increased, but the center of the defect was not filled, and the boundary between the defect location and surrounding bone structure was blurred (Figure 1g, h). The cavity is similar to the surrounding cancellous bone structure.

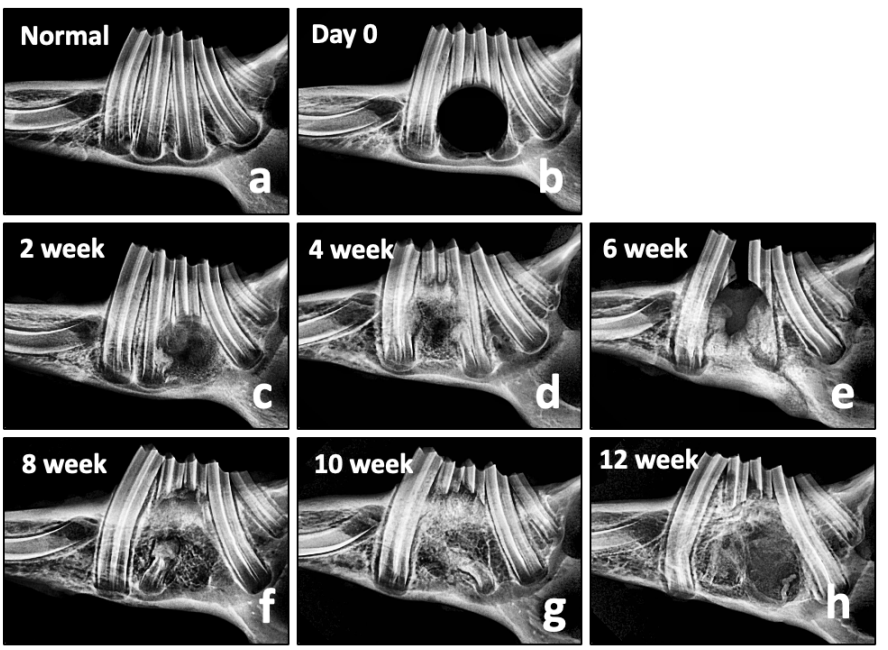

Figure 1. Assessment of the mandibular alveolar bone defect reconstruction process using X-ray
a. A photograph illustrates a typical rabbit mandibular alveolar bone.
b. On the first day, a deficiency in the rabbit mandibular alveolar bone was established.
c, d, e, f, g, h. Regeneration of the mandibular alveolar bone after surgery occurred over 12 weeks, with assessments performed at 2, 4, 6, 8, 10, and 12 weeks postoperatively.

Assess the capacity for regenerating the alveolar bone in the defective region using cone beam CT

Figure 2 depicts a three-dimensional simulation of the mandible reconstruction process. The normal mandible has a flat outer bone, which is entirely removed, including both the inner and outer bone plates, to create a gap in the body of the mandible (Figure 2b). At 2 weeks, limited bone regeneration was observed at the edges of the defect, with clearly defined boundaries around the defect area. At 4 and 6 weeks, bone regeneration was more apparent; however, the defect boundary with the surrounding bone structure was partially blurred. From 8 weeks onwards, the defect was almost filled, and the boundary with the surrounding bone structure was almost indistinguishable, with a convex outer surface on the bone.

The results of the CT beam sagittal slices passing through the center of the defect area are depicted in Figure 3. After two weeks, there was an increase in contrast at the edge of the defect, which was lower than that of the surrounding bone. The boundary between the defect and the surrounding bone was also clear. At four weeks, the control group showed more obvious bone formation, with bone radiopacity equivalent to that of the surrounding cancellous bone. However, the defect was not filled, and the boundary between the defect and the surrounding bone was still clear. After six weeks, a new bone had formed, but it had not yet filled the defect. The boundary between the defect and the surrounding bone was partially blurred. From eight weeks onwards, the radiopacity inside the defect was equivalent to that of the surrounding bone, and the boundary between the defect grafted with bone powder and the surrounding bone structure was partially blurred.

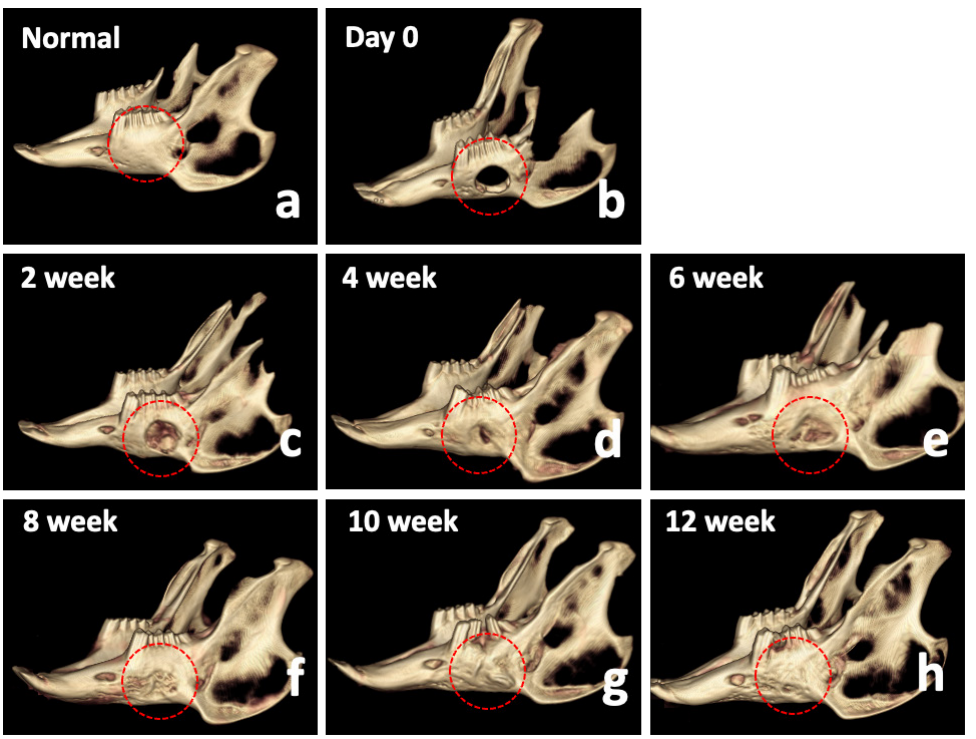

Figure 2. Three-dimensional images of the rabbit jaw were obtained using cone beam computed tomography (CT) scans at 2, 4, 6, 8, 10, and 12 weeks postoperatively

a. The 3D image shows a typical rabbit mandibular alveolar bone.

b. On the first day, a deficiency in the mandibular alveolar bone was identified.

c, d, e, f, g, h. Regeneration of the mandibular alveolar bone was observed using 3D pictures taken at 2, 4, 6, 8, 10, and 12 weeks postoperatively.

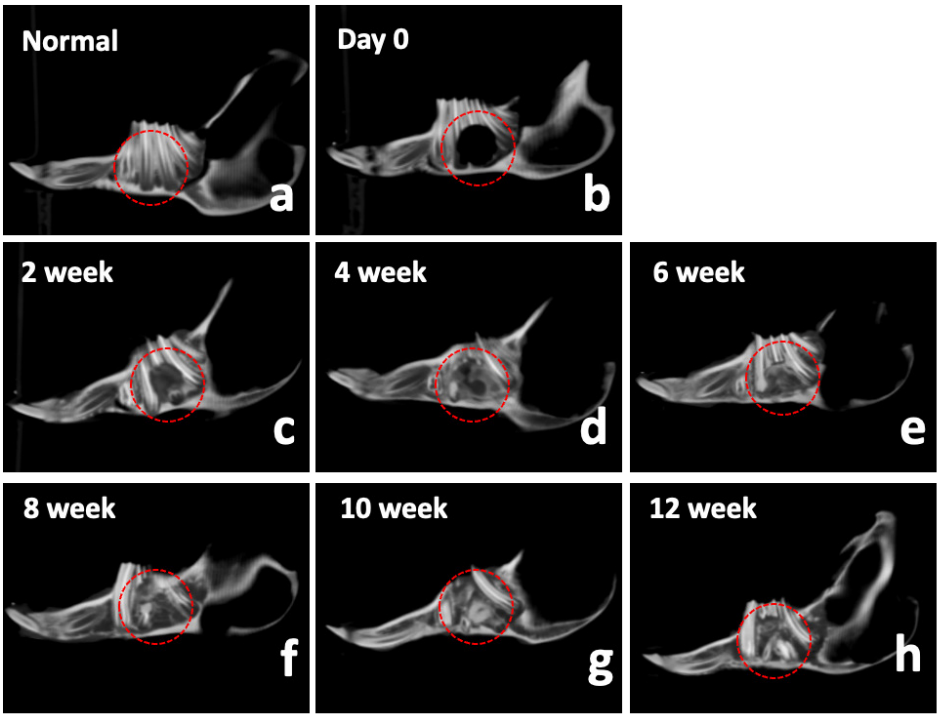

Figure 3. Cone-beam CT images in the sagittal plane of the rabbit mandible during the follow-up period.
a. A typical rabbit mandibular alveolar bone is shown in the picture.
b. On the first day, the rabbit had a mandibular alveolar bone deficiency.
c, d, e, f, g, h. The regeneration of the mandibular alveolar bone after surgery was evaluated 2, 4, 6, 8, 10, and 12 weeks after the operation.

The capacity of the defect area to regenerate alveolar bone was assessed through histological examination

Histological examination of the microscopic structure of the normal mandibular alveolar bone in rabbits revealed that it encompasses the tooth root and surrounding alveolar bone. Connective tissue, known as the periodontal ligament, is positioned between the alveolar bone and tooth root. At a magnification of 400x, bone cells were buried within the alveolar bone (Figure 4. a). After the creation of the defect, as shown in Fig. 4. b illustrates an area that does not contain the typical histological structure of alveolar bone. After two weeks, a significant amount of connective tissue had proliferated inside the defect (Figure 4. c). After four weeks, the quantity of connective tissue diminished, and alveolar bone began to form inside the defect, interspersed with the connective tissue (Figure 4. d). After six weeks, new bone started to form within the connective tissue. At a higher magnification, bone cells were buried within the newly formed bone cavities (Figure 4. e). From eight weeks onwards, the new alveolar bone began to form more clearly, replacing most of the connective tissue. Fewer connective tissue cells were observed inside the defect, and at higher magnification, many bone cells were buried within the bone cavities of the defect (Figure 4. f,g,h).

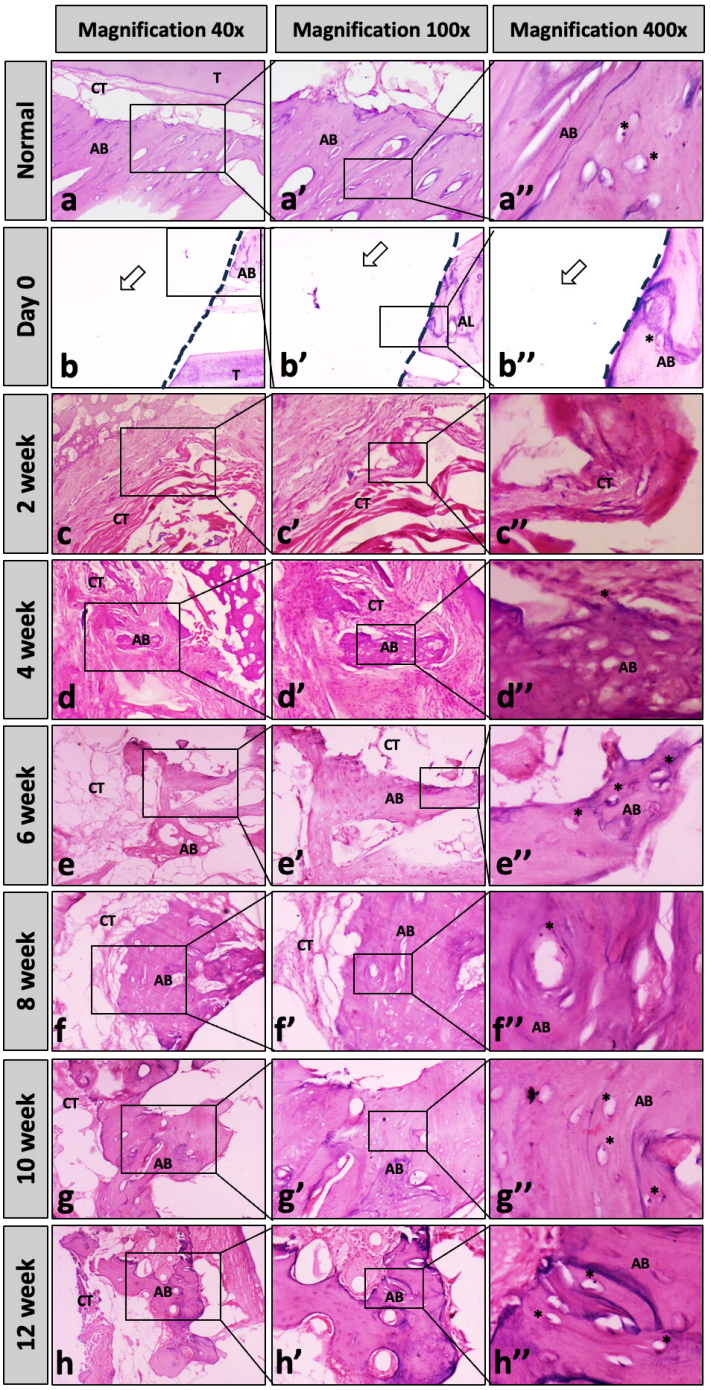

Figure 4. Histology of the normal mandibular alveolar bone and the model of mandibular alveolar bone defect regeneration 12 weeks after surgery

a, normal mandibular alveolar bone histological structure at different magnifications.

b, the mandibular alveolar bone defect was created.

c, d, e, f, g, mandibular alveolar bone regeneration after 2, 4, 6, 8, 10, and 12 weeks

T: tooth root tissue; AB: Alveolar bone; CT: Connective tissue; Asterisk: osteocyte; Arrow: defect area

4. DISCUSSIONS

Alveolar bone morphology defects are referred to as alveolar bone defects. These changes can be normal because of altered anatomical structures or pathologies [18]. Pathologies that result in alveolar bone defects include periodontal disease, trauma, maxillofacial tumors or cysts, surgery, and congenital causes [19]. The severity and location of the defect determine the clinical evaluation of the alveolar bone defect. Defects that compromise the structural integrity of bones can lead to impaired function of the masticatory system, which can negatively affect the patient's quality of life. For instance, a mandibular alveolar bone defect can impair a patient's ability to chew, swallow, or speak [20].

Most bone defects can heal on their own under the correct physiological conditions, as bones can regenerate themselves. Nevertheless, the healing process may take a considerable amount of time, and new bone formation may be slow due to limited blood supply to the fracture site, lack of calcium and phosphorus to strengthen and harden the new bone, and other factors, such as metabolic imbalances, hormonal fluctuations, nutritional deficiencies, and stress [21]. Furthermore, large defects may not heal spontaneously owing to factors such as the size of the defect, unstable biomechanical properties of the affected area, unfavorable wound environment, suboptimal surgical techniques, and other contributing factors [5].

The alveolar bone defect model involves creating a defect in either the upper or lower jawbone and using bone grafting materials such as autologous bone, allogeneic bone, and artificial bone powder to reconstruct the alveolar bone defect. This model is often used in clinical trials and interventions and is considered a suitable animal model for simulating alveolar bone defects [22].

Alveolar bone defects in animals can be created through surgical or congenital methods during pregnancy, as demonstrated in previous studies [23]. However, these methods have limitations. For instance, testing samples with congenital malformations in the mother's uterus necessitates the use of highly specialized techniques, and there is a high likelihood of associated fetal malformations, stillbirths, or miscarriages [24]. Furthermore, newborn animals with lip defects are often overlooked by their mothers and are at risk of being eaten; the resulting defects can vary in size and location [25].

Apart from the method of creating birth defects, defect modeling can also be performed using surgical methods. This approach is considered suitable for conducting experimental studies on the histological and biomechanical properties of bone graft materials [26]. Moreover, compared to congenital alveolar bone defects, surgical defect testing models in animals are easier to perform because they allow for control of the size and extension of the defect, as well as the location of the coated mucosa, which serves as the experimental sample [26].

The development of tissue engineering has led to the investigation of numerous regenerative biomaterials for the delivery of bioactive molecules to large bone defects. Initially, the safety and effectiveness of these materials were evaluated using in vitro systems, which are simple and can control the experimental variables. However, the interactions between multiple cell populations, growth factors, and underlying tissue in culture are intricate, making it challenging to replicate these interactions in vitro. Therefore, in vivo animal models are required for bone tissue regeneration studies [27].

To ensure the biocompatibility, mechanical stability, and safety of a new implant material before it is used in clinical settings, extensive testing must be performed under both in vitro and in vivo environmental conditions [28]. The use of appropriate animal models for preclinical testing is crucial for evaluating biocompatibility, tissue response, and mechanical function in various loading and unloading situations over extended periods, and under different biological conditions that are clinically relevant for new graft materials [28].

Animal models with simulated alveolar bone defects are commonly used as experimental models in clinical trials and interventions. These models include a range of animals, such as mice [12], pigs [8], rabbits [11], sheep [29], goats [30], dogs [31], and primates [32]. However, larger animals can be expensive to use for research because of the high cost of surgery and care, which limits the number of animals that can be included in studies. Therefore, rodents are often chosen as subjects for research on the applicability of biological materials because they have lower care and surgical costs [26].

The rabbit was selected for the study because of its alveolar bone in the molar and premolar regions, which measures 17 mm in length and 16 mm in height, providing an ideal space for creating a suitable defect and easy access to the surgical field [27]. Additionally, the rabbit diet has a good tolerance for mandibular defects, and it is

a non-aggressive, easily observable, and relatively inexpensive species that can be anesthetized and operated on with a large enough surgical field to insert grafting material [33]. The bone mineral content of rabbits is similar to that of humans, with a value of 2.49 ± 0.14 g/cm3 in humans and 2.51 ± 0.10 g/cm3 in rabbits [34]. Compared to other mammals, such as mice, pigs, and rabbits, rabbits have a higher bone metabolism rate and shorter bone regeneration cycles, with cortical bone as the main focus [35]. When creating facial bone defects in rabbits without bone grafting, micro-CT, and histological images showed complete bone healing without any signs of previous surgical intervention after nine weeks [36].

In 2013, Bölükbaşı et al. carried out a study investigated the effectiveness of PRF combined with biphasic calcium phosphate (BCP) in promoting bone regeneration in sheep tibial defects. The results indicated that the group treated with PRF and BCP showed the highest rate of new bone formation, whereas the other groups, including those treated with BCP alone, PRF alone, and no grafting materials, did not show any significant differences in the results. During the follow-up period, the bone grafts in both the BCP and PRF + BCP groups resorbed over time; however, the difference between these two groups was not statistically significant. The addition of PRF to BCP appeared to have a positive impact on histological bone formation in tibial defects of sheep [37].

In 2019, Cakir et al. conducted a study using a sheep model to assess the efficiency of growth factors in the fibrin network for bone regeneration. These findings demonstrate that a novel material created by combining synthetic β-TCP bone powder with PRF can support stable new bone growth and a longer-lasting structure. Additionally, the biocompatibility of this graft material is high, making it a safe and effective supplementary material for bone regeneration procedures [13].

In 2019, Seek et al. investigated the effect of platelet-derived materials, specifically platelet-rich fibrin, on bone regeneration in dogs. The outcomes demonstrated that when Bio-Oss was combined with PRF to treat a defect, new bone formation was significantly enhanced [14].

In 2020, Mu et al. prepared sticky bone by using protein-based beef bone powder and injectable PRF. The study demonstrated that sticky bone made from injectable PRF and deproteinized beef bone powder encouraged early angiogenesis and bone formation, thereby promoting more rapid bone regeneration in a grafted rabbit sinus model. While the bone volume in the sticky bone group was not significantly greater, the histological structure was of superior quality [38].

5. CONCLUSION

In summary, our study demonstrated that a substantial mandibular alveolar bone defect can be effectively created in rabbits. This procedure is relatively straightforward and can be performed without the need for magnifying or microscopic instruments. Postoperative assessment methods can be used to track the progress of alveolar bone regeneration. This rabbit model of mandibular alveolar bone defects is ideal for investigating and testing novel biomaterials in the field of alveolar bone regenerative medicine.

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