Replacement of tibial ostectomies in sheep by a titanium scaffold associated to a poliaxial locking plate.

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1. ABSTRACT

The development of customised implants for bone regeneration in sheep represents a significant advance in the field of experimental orthopaedic surgery. In this study, implants with a porous scaffold adapted to the anatomy of the sheep tibia were designed and manufactured with the aim of promoting osseointegration and bone regeneration. Two implant models were used, one located on the lateral side and the other on the caudal side of the tibia, which were fixed using locked and unlocked screws, respectively.

Four rams underwent surgery and were subjected to clinical and radiological follow-up during the postoperative period. The formation of new bone tissue was evaluated using radiographic images, as well as the stability of the implants and the functionality of the operated limb. The results showed progressive bone regeneration around the scaffold, especially in implants placed in areas of greater bone density and better fixation, which promoted adequate functional recovery.

Key aspects such as the influence of scaffold porosity, biomechanical load distribution, and implant design on osteogenesis were discussed, comparing them with previous studies. The limitations of the current model were also analysed, highlighting the need to improve fixation systems and optimise the protocol and surgical approach.
This work provides preliminary evidence on the feasibility and efficacy of customised porous implants in sheep models, laying the foundations for future improvements in orthopaedic implants adapted to the specific biomechanical characteristics of bone.

Keywords: Scaffold, sheep, bone defects, tibia.

2. INTRODUCTION

Segmental bone defects remain one of the most formidable challenges in modern orthopedic medicine, primarily due to the inherent limitations of traditional treatment methods. Historically, limb amputation has been the standard approach for managing severe complications arising from major trauma, bone tumors, or serious infections (Haam et al., 2024). In response, ongoing experimental research is focused on developing innovative techniques that can enhance patient outcomes and quality of life by promoting more effective bone regeneration with fewer complications. This has led to the emergence of the "Limb Sparing" approach (Pérez et al., 2019).

2.1 DEFINITION AND CLASSIFICATION

Segmental bone defects are characterized by the loss of an entire segment of bone tissue, with a size and location that exceed the body's natural regenerative capacity (Aerssens et al., 1998).

2.1Classification by Etiology

Segmental bone defects can be categorized based on their underlying cause:

- Post-traumatic: These are the most common type encountered in veterinary practice and typically result from high-energy injuries such as falls from significant heights, traffic accidents, or gunshot wounds (Liu & Ma, 2010).

- Iatrogenic: These defects arise following surgical interventions, particularly after extensive resections performed to treat chronic infections (such as osteomyelitis), bone tumors, or complications from previous surgeries (Liu & Ma, 2010).

- Pathological: This category includes defects secondary to chronic bone infections, nonunion fractures (pseudoarthrosis), metabolic bone diseases, or neoplastic processes that compromise bone integrity (Liu & Ma, 2010).

- Prosthetic Replacement: In patients who have undergone joint replacement or implantation procedures, bone defects may develop after the removal of prosthetic material, especially in cases involving periprosthetic infections or implant failure (Liu & Ma, 2010).

2.1.2 Classification by anatomical location

Segmental bone defects can also be categorized based on their anatomical location, in addition to their underlying cause:

- Diaphyseal: These defects involve the diaphysis, or shaft, of long bones. Spontaneous bone regeneration in this region is particularly challenging due to relatively poor vascularization compared to other areas of the bone (Vidal et al, 2020).

- Metaphyseal: These affect the metaphyseal regions, located near the ends of bones and adjacent to the joints. While these areas benefit from increased vascularization, managing large defects here remains complex and difficult (Vidal et al, 2020).

- Epiphyseal: These are located in the epiphysis, or the ends of the bone that contribute to joint surfaces. Epiphyseal defects are the least common and are most often seen in young animals, sometimes involving joint-related pathologies (Vidal et al, 2020).

2.2 LIMB SALVAGE TECHNIQUES

The primary objective of limb salvage techniques is to preserve the affected limb, thereby allowing the animal to maintain an optimal quality of life and preventing the various consequences associated with limb loss. Over the years, a range of techniques has been described and progressively refined, moving towards more innovative and complex approaches. Despite these advancements, significant limitations remain common to all methods, with infection risk, mechanical failure, and insufficient osseointegration representing the most frequent complications.

2.2.1 Ilizarov Technique (Bone Transport)

The Ilizarov technique is based on the biological principles of bone development and employs distraction osteogenesis to regenerate bone tissue. This method involves gradually applying traction stress to living tissue, which stimulates a process of regeneration and new bone formation. The Ilizarov technique utilizes a multiplanar circular external fixator, allowing for corrections to be made without removing the device. Its main advantage lies in the ability to regenerate autologous bone, thus avoiding the need for bone grafts. However, treatment is prolonged and requires strict compliance and collaboration from both the animal and its owner. Common complications include infections at the pin insertion sites, pain, and discomfort caused by the external device (Ting et al., 2010).

2.2.2 Bone Allografts

This technique involves replacing the bone defect with bone tissue from a donor, thereby restoring bone continuity without the need to harvest bone from the patient and reducing associated pain. However, bone allografts are associated with a high incidence of immune rejection and infection due to the host's immune response. Furthermore, the availability of suitable allografts is often limited, which can restrict their clinical application (Vlasa et al, 2025).

2.2.3 Endoprostheses

Endoprosthetic reconstruction aims to replace bone defects with an implant, typically made of stainless steel or titanium, which serves as a mechanical substitute and enables rapid functional recovery. Although these materials are biocompatible, they do not

promote osteointegration, which may result in implant loosening, infection, and functional limitations. Such complications are particularly frequent in cases involving joint replacement implants (Aldlyami et al, 2005).

2.2.4 Masquelet Technique

The Masquelet technique is a two-stage surgical procedure designed to address segmental bone defects. The process begins with the implantation of an antibiotic-impregnated cement spacer into the defect. This spacer induces a biological response, leading to the formation of an active, vascularized membrane around the foreign material. Once this membrane has matured—typically after several weeks—the spacer is removed, and the bone defect is filled with a bone graft. The induced membrane plays a crucial role by guiding bone regeneration, stabilizing, and supporting the grafted tissue. The principal drawback of this technique is its requirement for at least two separate surgical interventions. Additionally, meticulous care is essential throughout the process due to the fragility of the induced membrane, which is vital for successful bone integration and healing (Apard et al, 2010).

2.2.5 Vascularized Ulnar Transposition

Vascularized ulnar transposition is most commonly used to reconstruct bone defects in the distal portion of the radius. This technique involves transferring a segment of the ulna, along with its blood supply, to the site of the defect. Because the graft is autologous, it promotes better integration and significantly reduces the risk of infection. Another advantage is that it typically requires only a single surgical procedure. However, this method is limited to specific types of defects and anatomical locations. Furthermore, harvesting the ulnar segment may result in donor site weakness, which must be considered when selecting candidates for this technique (Gundavda et al, 2021)

2.3 POROUS TITANIUM SCAFFOLDS

2.3.1 Osseointegration

Porous titanium scaffolds represent one of the most significant advances in tissue engineering for the reconstruction of bone defects. Titanium is highly biocompatible, with excellent corrosion resistance and favorable mechanical properties, making it the material of choice for many orthopedic procedures in both veterinary and human medicine.

The primary objective when placing these implants is to achieve successful osseointegration, meaning that bone tissue forms a direct and stable bond with the surface of the implant, without the presence of intervening fibrous tissue. A distinctive feature of these scaffolds, compared to earlier implants, is their porosity. This property encourages cell migration, vascularization, and the transport of essential elements required for new bone formation. Additionally, the scaffold's structural similarity to the natural porosity of bone provides the necessary mechanical stimulation for bone regeneration, while minimizing stress-related complications associated with excessively rigid implants (Wieding et al., 2015)

2.3.2 Clinical Advantages and Application in Veterinary Medicine

Studies and animal models have demonstrated that patients are able to bear weight at an early stage following surgery. This represents a significant advantage over other methods, as it markedly reduces complications associated with postoperative muscle atrophy. Furthermore, given the variability in size and weight among veterinary patients, the ability to design custom implants for each species and individual case allows for more effective management of complex reconstructions in anatomically challenging regions (Crovace et al., 2020).

2.3.3 Ti6Al4V Alloy

Ti6Al4V is currently the most widely used titanium alloy worldwide. Its composition consists of 90% titanium as the base element, 6% aluminum, and precisely 4% vanadium. This formulation provides a biphasic α-β structure, which imparts strength, thermal stability, ductility, and deformability. The alloy is highly biocompatible and exhibits a high capacity for osseointegration (Gatto et al., 2021)

2.3.4 Material fusion techniques, EBM and SLM

Electron Beam Melting (EBM) is a material fusion technique involving the layer-by-layer deposition of metal powder, which is selectively melted using an electron beam as the heat source. This process is performed in a vacuum, preventing material contamination and oxidation. This process operates at elevated temperatures, 700-1000°C, which allows processing materials with high thermal conductivity and high melting point (Gibson et al, 2015).
Selective Laser Melting (SLM) is another material fusion technique that employs a high-powered laser to melt metal powder in thin layers according to the printing code, thereby producing highly precise components. This occurs in an inert gas environment, typically argon or nitrogen, and lower temperatures than in EBM are used, up to 300°C of preheating (Gibson et al, 2015).

2.4 EXPERIMENTAL ANIMAL MODELS

In recent years, a range of studies has explored limb salvage techniques across various animal species, including sheep, rodents, dogs, and horses. Much of this research is motivated by the potential for application in human medicine, which is why sheep are frequently selected as the preferred model. Adult sheep share similar body weight and bone characteristics with humans, making them especially suitable for these studies.
Sheep also offer practical advantages: they are easy to manage and maintain, and their lifespan is long enough to support extended studies. This allows researchers to monitor implant integration and behavior over different phases of bone regeneration. From an ethical standpoint, using sheep is generally more acceptable than using companion animals, which facilitates preclinical research.

2.4.1 Anatomy

The sheep tibia is notably robust and well-adapted to withstand the dynamic forces experienced by quadrupeds. Measuring approximately 30 cm in length, with a diameter of around 24 mm and a cortical thickness of 4 mm, it is frequently used in orthopedic research. Its continuous bone remodeling and well-developed Haversian systems make it an excellent model for studying bone regeneration and osseointegration.

When planning surgical approaches, it is important to consider the various anatomical structures attached to the bone, including muscles and tendons, as these contribute to limb stability, articulation, and movement. In sheep, as in most species, the medial surface of the tibia functions as the tension side. This area is flat and readily accessible during surgery due to minimal muscle coverage. Muscles such as the gracilis and semimembranosus, which are involved in flexion and abduction, as well as the popliteus, which aids in knee stabilization and internal rotation, attach here.

Conversely, the lateral surface is the attachment site for muscles like the tibialis cranialis and the peroneal group, which can limit surgical access and complicate plate fixation. The cranial aspect of the tibia is slightly convex, while the caudal side is broader and concave. Although the caudal surface is easily accessible, the presence of strong muscles such as the gastrocnemius, soleus, and digital flexors can interfere mechanically with implants (Sisson et al., 2001).

3. OBJECTIVES

The objectives of this project are:

- Repair of the bone defect with a scaffold and an osteosynthesis plate.
- To verify the union of the bone during recovery.
- To determine the mechanical properties of the healing.
- Histomorphometric evaluation of the bone and scaffold. Analysis of the orientation of collagen fibres and mineralisation in the bone structure

4. MATERIAL AND METHODS

4.1 ANIMALS

Data obtained from four experimental clinical cases in sheep patients between March and April 2025 at the Clinical Veterinary Hospital of the University of Las Palmas de Gran Canaria were included in this study. All subjects selected were Pelibuey males, aged between 4 and 7 years and weighing between 38 and 47 kg. The study was performed on the left hind limb in all patients.

Identification	Date	Race	Sex	Weight
47212	20/03/2025	Pelibuey	Male	50 Kg
41784	10/04/2025	Pelibuey	Male	45 Kg
No identification	10/04/2025	Pelibuey	Male	45Kg
71785	24/04/2025	Pelibuey	Male	47 Kg

These animals come from the farm of the veterinary faculty of the University of Las Palmas de Gran Canaria. All experimental procedures with animals were carried out in accordance with Directive 2010/63/EU and Royal Decree 53/2013 on the protection of animals used for scientific purposes. The protocol was evaluated and approved by the Animal Experimentation Ethics Committee (CEEA) of the ULPGC and authorised by the competent authority (reference/approval code: OEBA_ULPGC 16/2024). https://www.ulpgc.es/vinvestigacion/ceea.

4.2 FABRICATION AND DESIGN OF THE IMPLANTS

This project was carried out in conjunction with engineers from the Instituto Tecnológico de Canarias (ITC), together with engineers from the department of mechanical engineering of the University of Las Palmas de Gran Canaria.

The engineers needed 3D images of the tibia morphology of the study subjects in order to develop implants adapted to each patient's anatomy. Therefore, between June and July 2024, the animals underwent computed tomography (CT) scans under anaesthesia.

The anaesthetic protocol used began with premedication of the patient, in which Xylazine (Sedaxylan®) was used at a dose of 0.05mg/kg, intramuscularly. This drug is an α2-adrenergic agonist, which provides sedative, analgesic and myorelaxant effects to the patient, allowing a more affordable management, avoiding defensive reactions and reducing the animal's stress. Approximately 10 minutes after premedication, when signs of the effect of premedication were already visible in the patient, the rams were prepared for placement of an intravenous catheter in the distal cephalic vein. Anaesthesia was induced with intravenous Propofol lipide (PropoVet®) at a dose of 1mg/kg, a fast-acting hypnotic agent that allowed a smooth and controlled loss of consciousness.

Because CT is a brief and non-invasive procedure, anaesthetic maintenance was performed with Propofol Lipide as the hypnotic agent, which benefited the animal with a rapid anaesthetic recovery.

Once the necessary images of the tibia of the animals were obtained, the development of the prostheses began. The prostheses are composed of 3 elements, each with a specific function: a cutting guide, a plate containing the porous implant (scaffold) and an osteosynthesis plate. The latter two were designed to adapt to the lateral and medial aspect of the tibia, respectively.

These prostheses are made of $_{Ti6Al4V}$, a titanium alloy (90%) containing aluminium (6%) and vanadium (4%). The implants were fabricated on a titanium 3D printing machine using electron beam (EBM) additive manufacturing.

Cutting guide

The cutting guide is an element that facilitates the surgeon to make the cuts in the exact direction, angle and location to complete the ostectomy accurately. In this case, the aim is to achieve a bone defect that fits the porous implant, ensuring that there is intimate contact with the bone to promote osseointegration. This guide was designed to adapt to the medial side of the tibia, it has two extensions that project towards the lateral side, with the aim of increasing contact with the bone surface, improving stability and positioning precision during cutting.

Plate with porous structure (Scaffold)

There were two designs of this plate containing the porous implant (scaffold), which is prepared to fit the defect generated with the ostectomy, acting as a bone substitute and favouring bone regeneration. The scaffold is not designed to be primarily load-bearing.

The first model was adapted to the lateral aspect of the tibia and had the scaffold separated from the plate by appendages. It had four holes for screw placement, two at each end of the implant. Both the most proximal and the most distal hole with respect to the bone diaphysis are compression holes and are designed for cortical screw placement. The remaining holes, closer to the scaffold, are designed for the placement of dynamic compression locking screws.

The second implant was designed to be placed on the caudal surface of the tibia due to the enormous difficulty of placing it on the lateral surface. In addition, the number of screws was reduced because the project's chief engineer preferred that the scaffold not bear loads.

In both models the scaffold portion is the same, with no modifications in design, size or porosity. These models have a diameter of 20.01mm, a three-dimensional volume of 6cm3, a weight of 5.589 grams and a porosity of 80.89%.

Osteosynthesis plate

As mentioned, the scaffold is not primarily load-bearing, so an osteosynthesis plate is added to this technique on the medial side of the tibia (tension side). Its function is to redistribute the biomechanical loads, relieving the stress that would otherwise be placed on the scaffold. It is a support plate with eight holes for the placement of locking screws, four at each end of the plate, which interlock with the screws used in the fixation of the porous implant plate, thus providing greater structural stability. The central part is the part that will bear the most weight and is marked with the position of the porous graft.

In addition, a specific surgical kit was developed for the fixation of these implants, created with the same titanium alloy ($_{Ti6Al4V}$). This material included locking screws and 2.7mm and 3.5mm cortex screws, together with their respective drill bits and drill guides, as well as depth gauges and compatible screwdrivers.

Fig 1. Cutting guide.

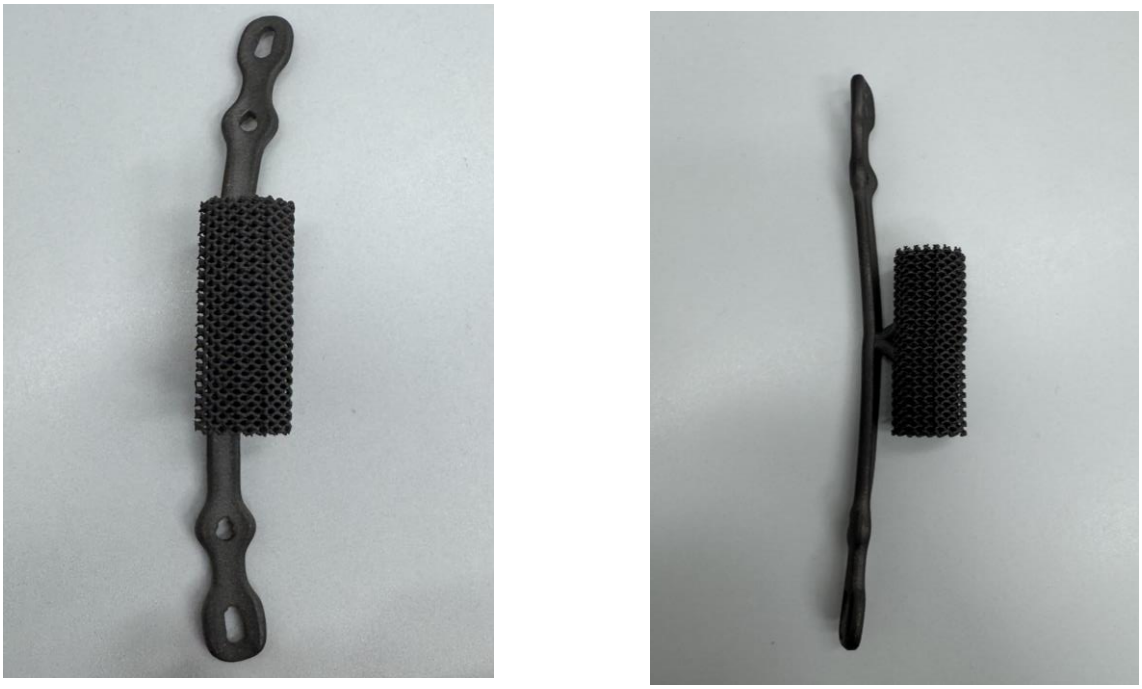

Fig 2. First model of scaffold.

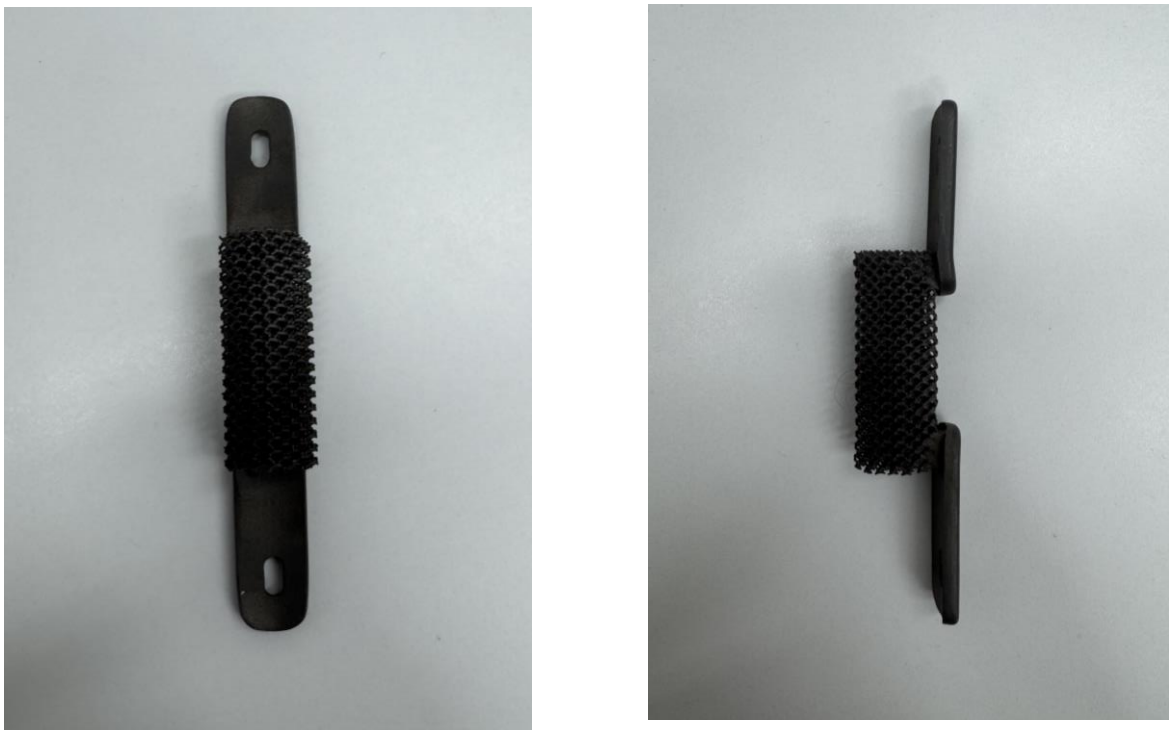

Fig 3. Second model of scaffold.

Fig 4. Osteosynthesis plate.

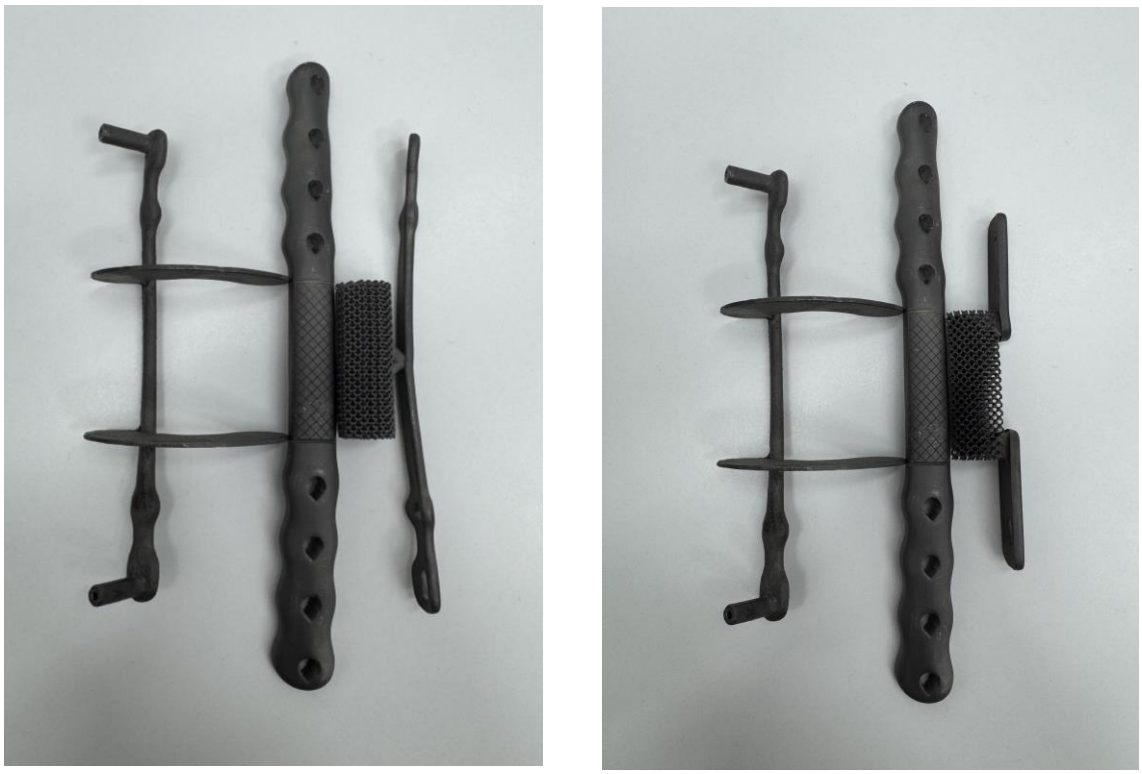

Fig 5. These images show the coincidence between the section of the cutting guide used to create the bone defect, the portion of the osteosynthesis plate corresponding to that defect, and the scaffold, both for the first and second models respectively.

4.3 SURGICAL PROCEDURE

For surgical anaesthesia, the same premedication and induction protocol was used as for CT, Xylazine (Sedaxylan®) at a dose of 0.05mg/kg, intramuscularly and induction with intravenous Propofol lipide (PropoVet®) through a catheter placed in this case in the distal saphenous vein of the limb that was not going to undergo surgery.

In this case, a deep anaesthetic plane was required due to the fact that the patients were to undergo surgery, so anaesthetic maintenance was carried out with sevofluorane vaporised in 100% oxygen, using a closed-circuit inhalation anaesthesia system. For this, endotracheal intubation of the patient was necessary, as well as an orogastric tube. This last step is essential in a general anaesthesia, since this species is prone to abdominal distension when in lateral or sternal decubitus due to the accumulation of gases in the rumen that cannot be eliminated via the oesophagus. The catheterisation allows emptying of the rumen and evacuation of gases, reducing the risk of abdominal distention and regurgitation.

In addition, a locoregional block was performed to improve analgesia and achieve greater stability during anaesthesia and postoperative recovery. The sciatic and saphenous nerves were blocked with two different anaesthetics. Lidocaine (Lidor®) (used in the first patient) at a dose of 1mg/kg and Bupivacaine with a concentration of 0.5mg/ml at a dose of 1mg/kg (infiltrated in the last three patients), infiltrated in the corresponding anatomical points with the help of ultrasonography.

4.3.1 Surgical technique

The patients were positioned in lateral decubitus, with the limb that was to undergo surgery fully extended and resting on the surgical table, leaving a medial view of the tibia. Once the animal was correctly positioned, the medial approach to the tibial diaphysis was performed, consisting of a longitudinal skin incision along the palpation area of the diaphysis, from the knee region to the medial malleolus area.

Using blunt dissection, the subcutaneous planes are dissected and, after incising the crural fascia, the cranial tibialis and extensor digitorum longus muscles are moved laterally and the flexor digitorum profundus caudally. This exposes almost the entire tibial diaphysis.

The first step in this technique is the correct positioning of the cutting guide. This is adjusted on the medial side, leaving the portion where the ostectomy is to be performed in the middle third of the bone. To avoid displacement, it is provisionally fixed with K-wires (1.5 mm). The cut is made with an oscillating saw, irrigating with sterile saline during the procedure to avoid thermal necrosis, which would compromise bone regeneration.

Once the bone defect has been created, the porous implant is placed. In this step it is important that both bone surfaces have adequate contact with the scaffold to promote osseointegration. As mentioned above, there were two models of this plate, the first one is fixed on the lateral side of the bone, while the second model is fixed on the caudal side.

When performing the first procedure using the first model of the implant, the tibia is approached from the lateral side by lifting the cranial tibial muscle, which makes it considerably more difficult to adapt the plate and drill the holes for screw placement. This increases both the surgeon's discomfort and the patient's anaesthetic and surgical time, which was increased by more than an hour compared to the placement of the second implant model. Therefore, only one single operation was performed with this model before the plates were redesigned. The fixation of this implant was carried out with two 2.7 mm locking screws in the holes closest to the scaffold, and two 2.7 mm cortex screws in the remaining holes, inserted under compression.

With the second model, three interventions were performed, adjusting the scaffold in the same way in the bone defect formed, but in this case fixing the plate on the caudal side of the tibia, using two 2.7 mm cortex screws. There were no complications in any case.

Once the porous implant plate is correctly positioned and fixed, an osteosynthesis plate is placed on the medial aspect of the tibia to redistribute the biomechanical loads and minimise stress on the scaffold. For the placement of this plate, the section marked on the plate must be correctly adjusted to the position of the scaffold, so that no screws come into contact with the fixation screws of the porous graft. Eight 3.5 mm locking screws were used.

Finally, all the implants were checked to ensure that they were correctly positioned and fixed, as well as the correct alignment of the animal's limb. As a final step, surgical closure is performed in planes with resorbable suture.

4.3.4 Postoperative Treatment

Subsequently, after surgery, a multimodal analgesia protocol was implemented with the aim of controlling pain, preventing infections and favouring an adequate recovery. Meloxicam (Canidolor®) was administered as a non-steroidal anti-inflammatory drug at a concentration of 5mg/ml at a dose of 0.2 mg/kg subcutaneously to manage pain and inflammation. Oxytetracycline (Oxytetracycline invesa®) at a concentration of 200mg/ml at a dose of 20mg/kg subcutaneously was used as an antibiotic as a prophylaxis against possible infections. Both drugs were administered in single doses after surgery.

The follow-up plan for these animals consists of three months of clinical monitoring, during which the functionality of the operated limb and the patient's pain level are assessed. Once this period has elapsed, an X-ray is taken to assess the progress of bone healing, and the patients are euthanised so that the tibia can be removed and samples taken for histological testing.

5. RESULTS

In the present study, four surgeries were performed as follows: one surgery using the first scaffold model and the other three with the other model, which had been previously redesigned based on the results of the first surgery. A previously established protocol was followed with all cases, which consisted of three fundamental steps: surgery, immediate post-surgical radiography and radiological control three months after the intervention. A clinical follow-up of the signs of each patient was also carried out, assessing the functionality of the limb.

CASE 1:

In the first case, the first model designed was used. The X-ray immediately after surgery showed correct fixation of the implants, although due to the difficulties mentioned above, the plate was not perfectly aligned on the lateral side as expected, but showed a slight caudal deviation.

Over the course of the weeks, the patient gradually began to support the limb and began to bear weight on it. After 3 months of follow-up, the patient had fully regained function of the limb and showed no signs of pain or apparent lameness. A control X-ray was taken to assess the evolution of the patient. These X-rays show abundant bone tissue formation, which is evident bone tissue formation is evident along the entire length of the defect created, highlighting that on the caudal side of the bone the neoformation is greater, completely enveloping the scaffold.

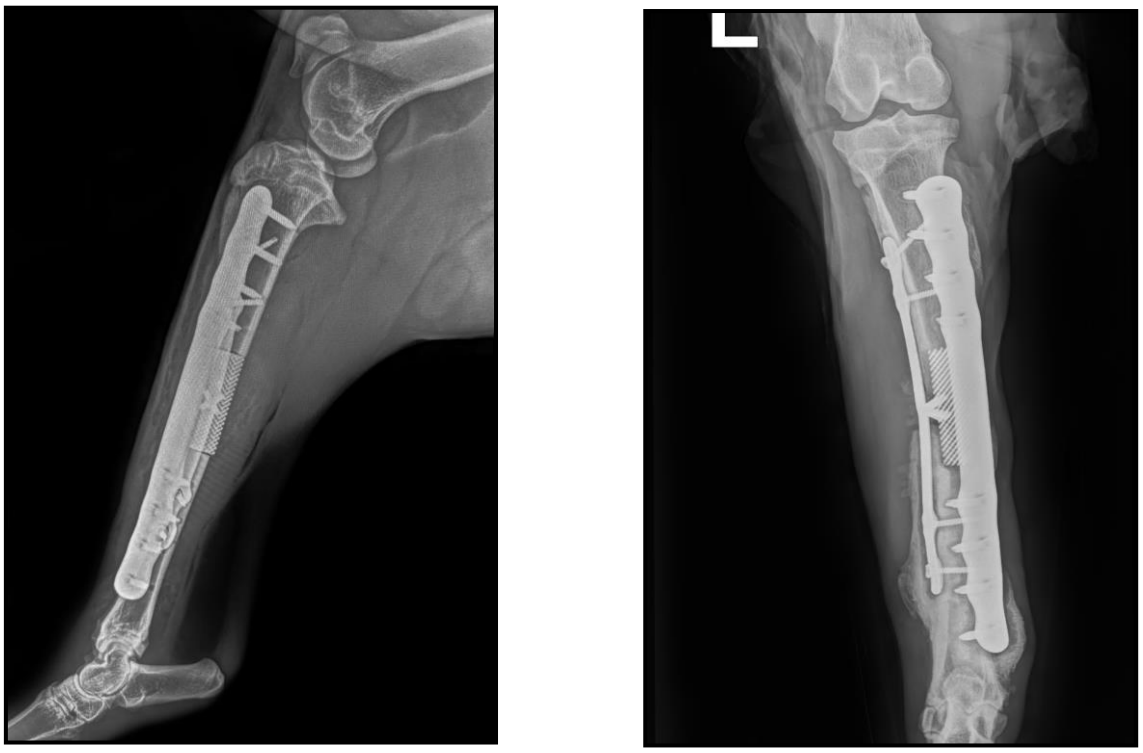

Fig 6. Immediate X-ray after surgery of the first patient, first implant model is used.

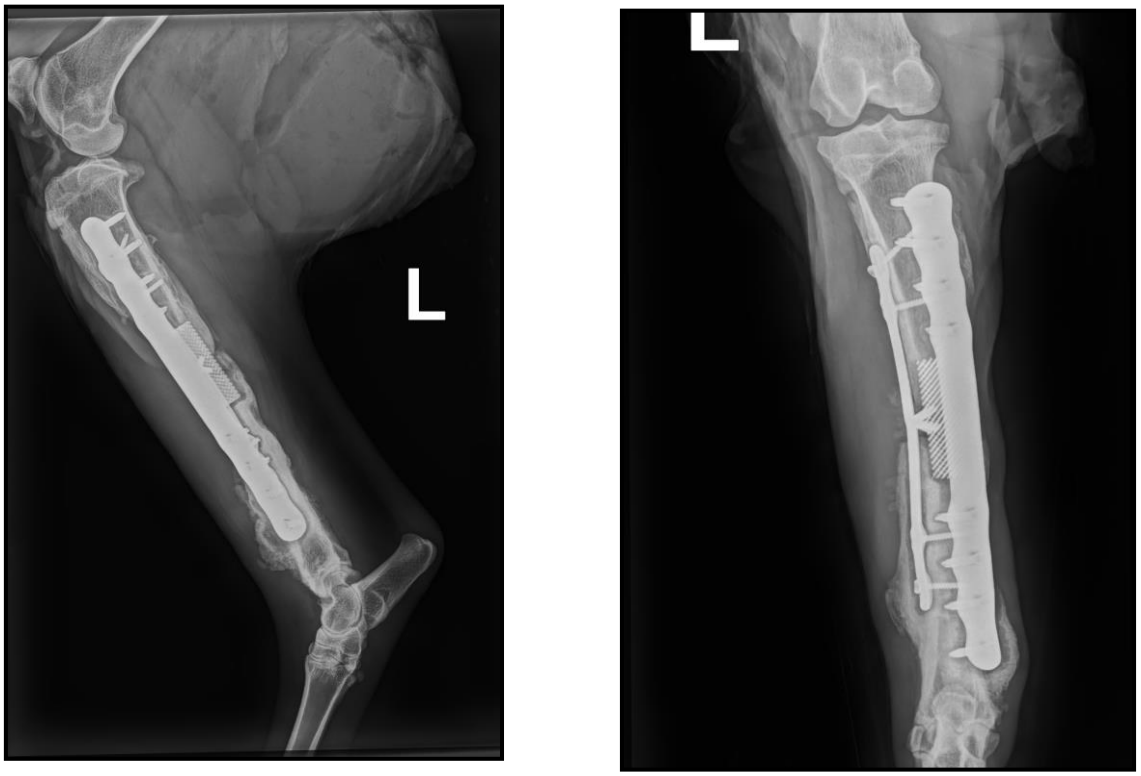

Fig 7. Radiological evolution 3 months after surgery of the first patient.

CASE 2:

The second implant design was used, and the same clinical and radiological follow-up was performed as in the first case. The X-ray taken immediately after surgery shows correct alignment of the limb in its physiological form, but during recovery, the implant shifted medially, causing valgus, reducing support and use of the limb. This led to a less favourable outcome for the patient compared to the first case. Recovery was slower, and full limb function was not regained; three months after surgery, the patient still had a limp and reduced weight-bearing capacity. However, the follow-up X-ray showed bone tissue formation, mainly on the lateral side of the defect. Compared to the first model, this neoformation was delayed, although it remained favourable.

CASE 3 AND 4:

Initial fixation was optimal in all patients, with the plates perfectly aligned medially (osteosynthesis plate) and caudally (scaffold plate). The subjects were clinically monitored again. However, in both patients, the plate containing the porous graft fractured, one immediately after surgery, upon awakening from anaesthesia, and the other on the third postoperative day. These animals were humanely euthanised, they were previously sedated with xylazine (Sedaxylan®) and subsequently was administered intravenous administration of phenobarbital (Euthoxin®).

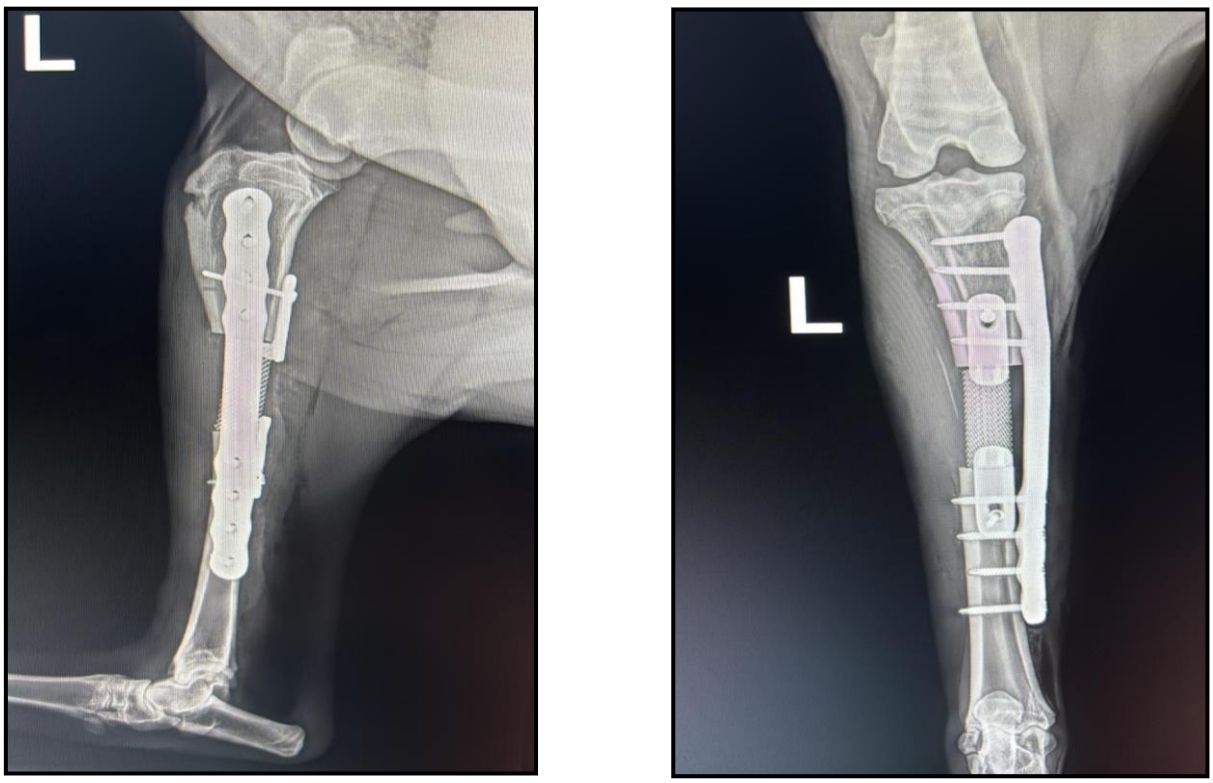

Fig 8. Immediate X-ray after surgery of the second patient, second implant model is used.

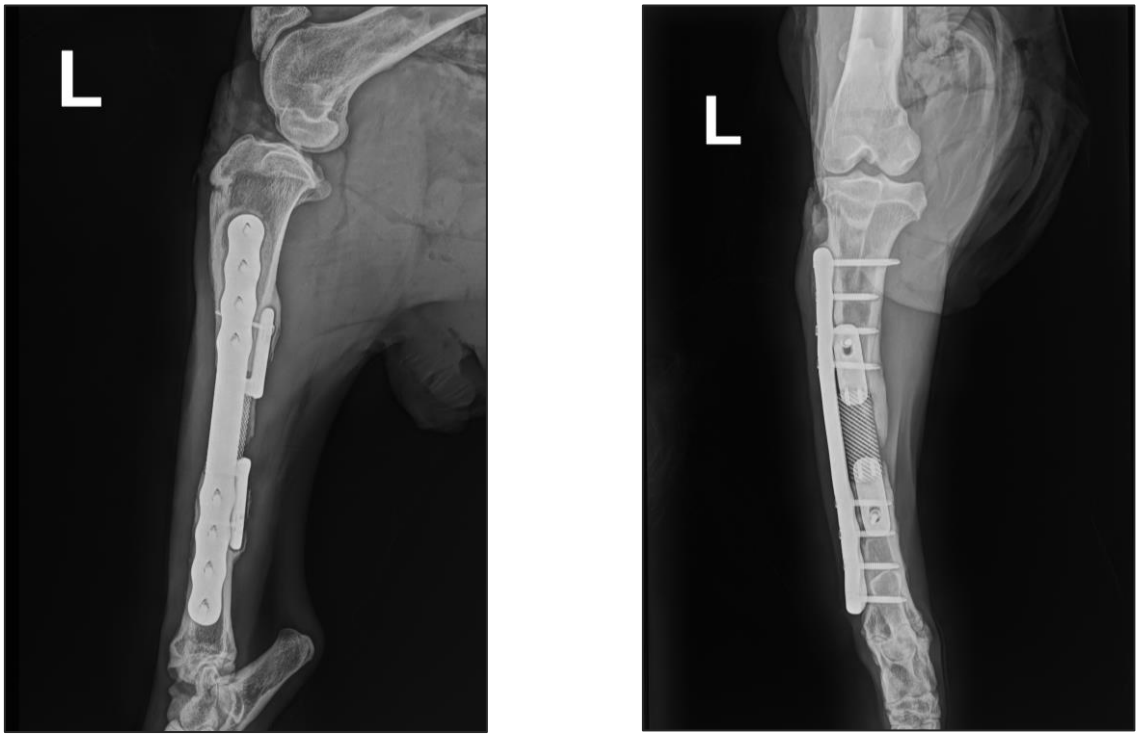

Fig 9. Radiological evolution of the second patient 3 months after surgery.

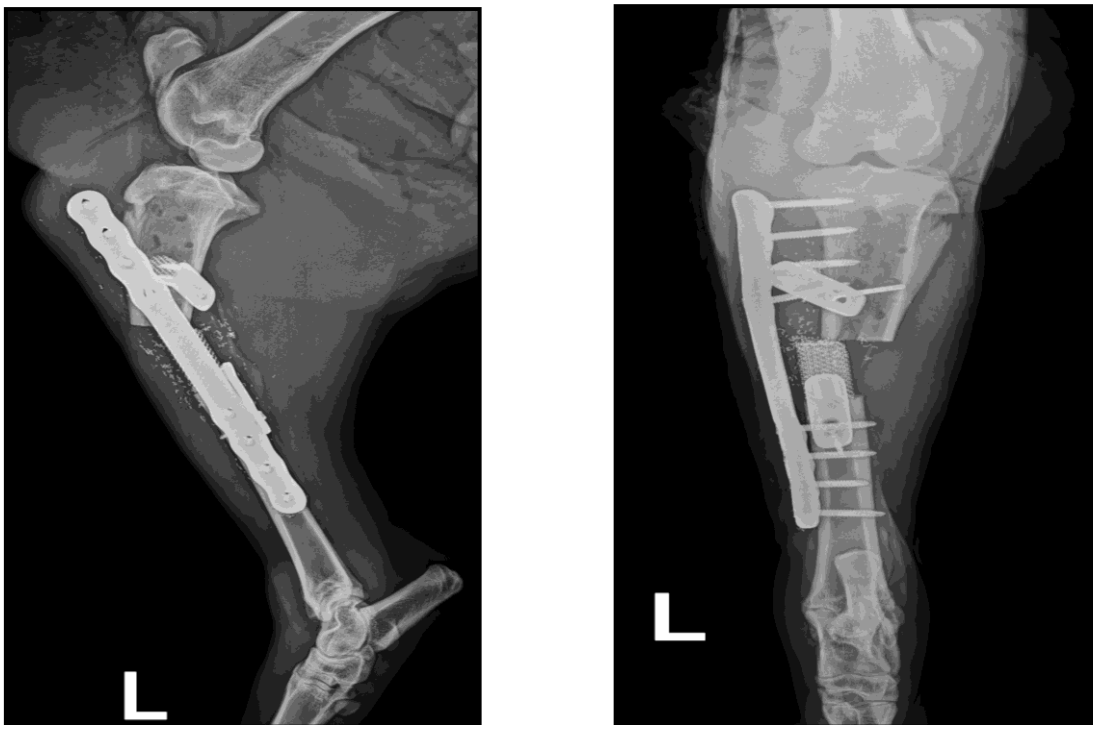

Fig 10. X-ray of the third patient 3 hours after surgery.

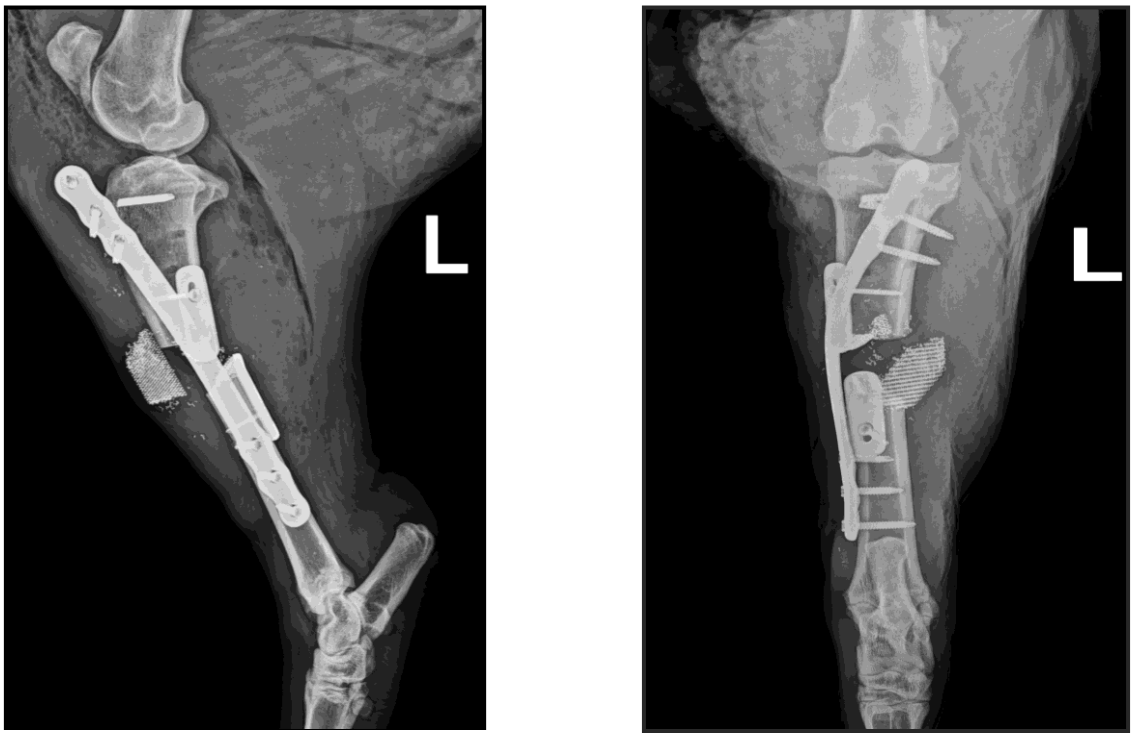

Fig 11. X-ray of the fourth patient 3 days after surgery.

In the radiological review three months after the operation of both patients, osteogenic activity can be seen around the porous graft, however, no radiopaque bone bridges can be seen connecting the new bone tissue with the porous graft, giving structural continuity between the tissues. This is reflected by the presence of a radiolucent area between the scaffold and the new tissue.

It is common that a simple X-ray cannot accurately assess the contact between the porous implant and the bone neoformation after three months of evolution. In order to be able to assess whether the relevant bone bridges are actually forming and are the beginning of osseointegration, histological tests of the tissue must be carried out to evaluate the progress at the histological level.

After evaluating the patients' progress over three months, the animals were euthanised, they were previously sedated with xylazine (Sedaxylan®) and subsequently was administered intravenous administration of phenobarbital (Euthoxin®). The animals were euthanised in order to remove the tibia and freeze it pending the dispatch of samples for histological testing, which will be carried out in the coming months.

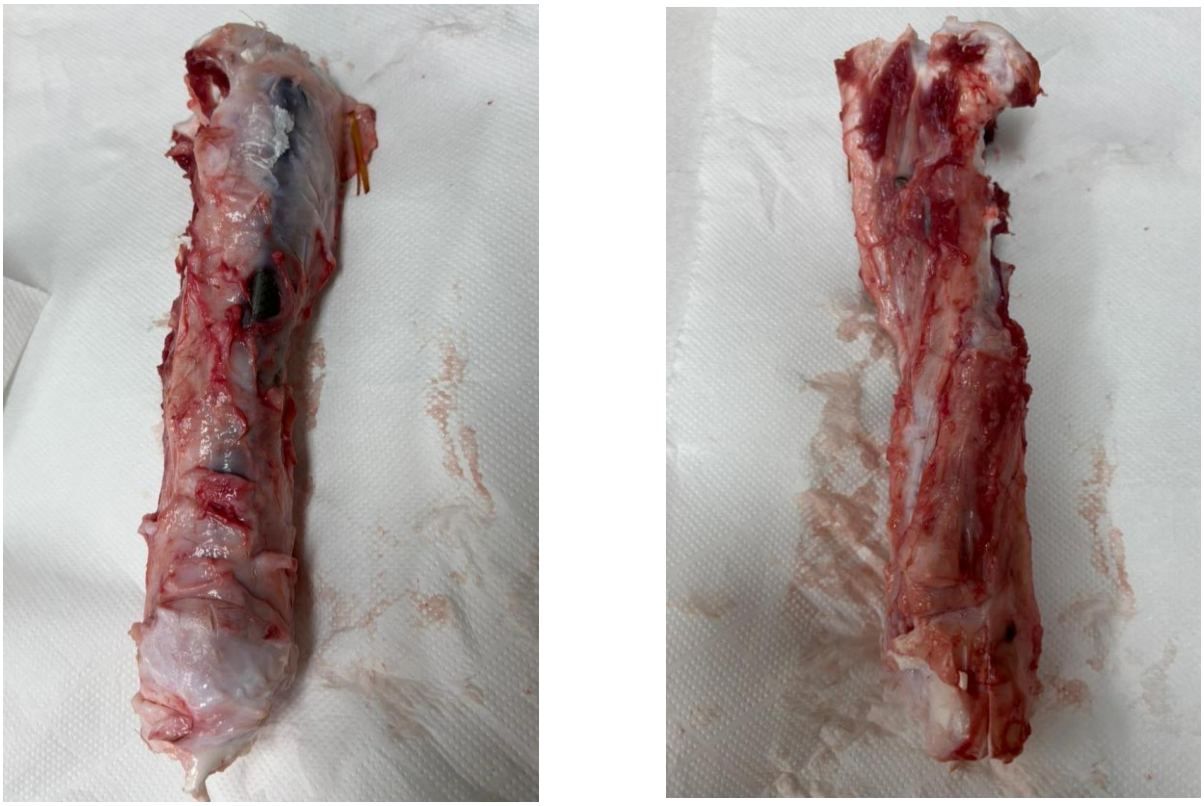

Fig 12. Dissected tibia of the first sheep, viewed from the cranial and caudal sides, respectively.

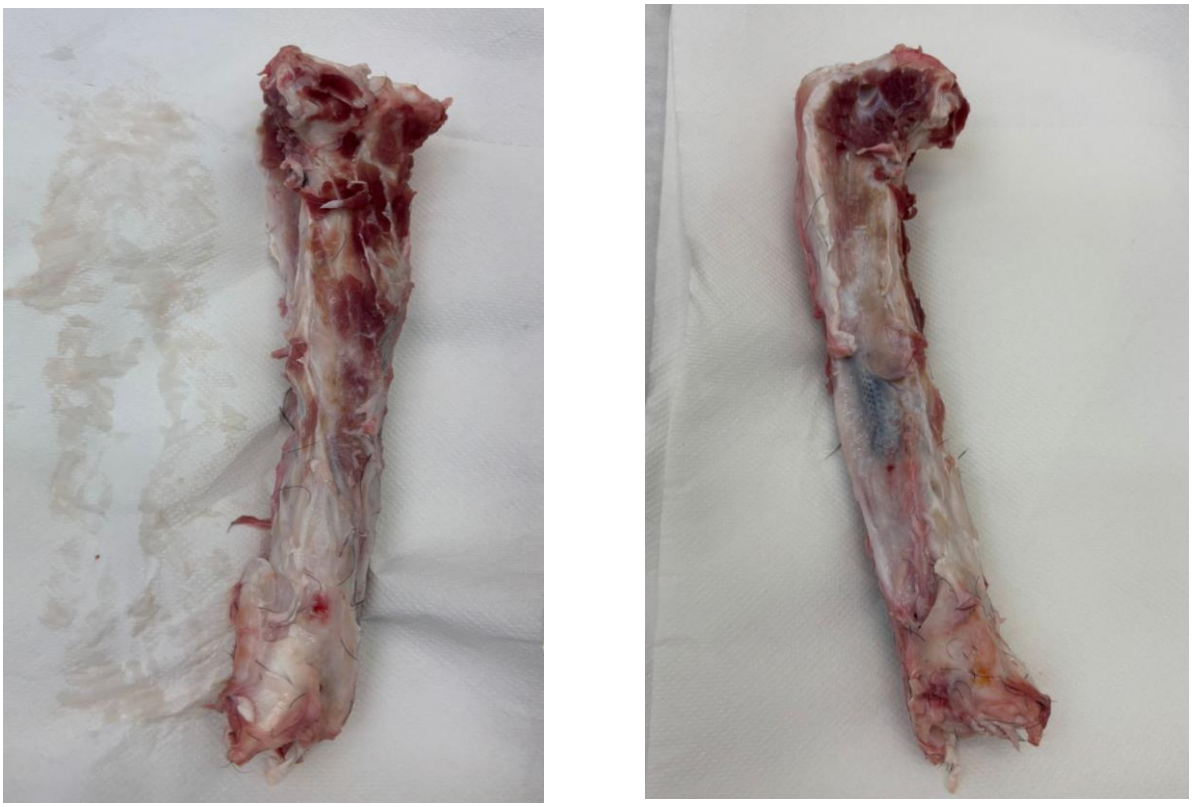

Fig 13. Dissected tibia of the first sheep, viewed from the cranial and caudal sides, respectively.

6. DISCUSSION

One of the most important parameters for the design of implants used in bone regeneration is porosity. Recent studies have shown that high porosity (>60%) favours cell migration, nutrient transport and vascularisation, which promotes proper osteogenesis. However, it should be borne in mind that excessive porosity can compromise mechanical properties, such as compressive strength (Pérez Sánchez et al., 2025).

In contrast, low porosity (<60%) has been found to improve the mechanical properties of the scaffold, but limits cell proliferation, making it less interesting from the point of view of the osseointegration sought with this type of implant (Velasco Peña et al., 2023). In the present study, a scaffold was used with a porosity of 80.89%, high porosity which, as mentioned, has both benefits and drawbacks.

The failures obtained in the present study are not an isolated phenomenon in the literature; previous studies have been carried out in sheep with scaffolds made with Ti- 6Al-4V that coincide with our observations. In these studies it can be seen that even in well designed and implanted scaffolds, the balance between porosity and structural strength can lead to failure. A study with tibial defects in 20 sheep was conducted in July 2023, in which all animals were sacrificed within the first month after placement of the porous graft due to bending of the plates due to mechanical overload (Wieding et al, 2015; Yongfeng Li et al., 2023).

However, the success of this type of implant can also be seen in other studies. In a study using Ti-6Al-4V scaffolds manufactured by EMB, as is the case in the present study, and a porosity of 90%, the results were very favourable. The animals had an almost immediate functional recovery after surgery, and twelve months after the intervention, a complete integration of the scaffold is seen. In addition, the formation of bone bridges between the porous graft trabeculae was corroborated by histology. This ensures that EBM-synthesised titanium implants are able to bear weight and have good osseointegration with the recipient (Crovace et al, 2020).

The success of this trial validates that the incident in two of the cases in this study, in which the scaffold plate fractured, is not due to the material used for its fabrication or the high porosity of the graft, as favourable results have been seen with similar data. However, the fixation system used, together with the anatomical position of the implants, may have been key to these events.

Initially, implants were designed to be placed on the lateral aspect of the bone, but this approach brought with it a number of problems, such as difficult access to this area and the possibility of causing damage to neurovascular structures, which can lead to ischaemic muscle necrosis due to injury to the common peroneal nerve and anterior tibial artery, resulting in skin loss. In addition to limited exposure that compromises the correct reduction and fixation of implants (Chen & Luo, 2015).

These reasons led to the decision to redesign the scaffold plate for placement on the caudal aspect of the bone, where the approach is less complex and relevant anatomical structures are not compromised. It has been shown in the scientific literature over the years that the use of orthogonal plates (90-90 degrees) provides high defect stiffness and greater biomechanical stability compared to parallel plate placement. The use of this system is the most suitable for dealing with the forces to which a bone defect is subjected, the ideal is to place two orthogonal plates that will be placed on the cras of the bone that are directly subjected to the forces (Patel et al, 2021). In the present study, the medial-caudal configuration was chosen because the cranial aspect of the tibia in sheep has a marked tibial ridge which makes it difficult to adapt the implants (Sisson et al, 2001).

Although the problems mentioned above have been solved, the existing literature shows that sheep do not have the same bone density on all sides of the cortical bone due to the different loads that the bone physiologically undergoes. The medial side supports 75% of the axial load of the bone, having a higher density than the areas where there is less mechanical load (Brzezinski et al, 2017, Gautier et al, 2000).

The lower density of this area makes it a less optimal option for plate fixation, as this condition causes it to have lower mechanical strength and reduces the anchorage capacity of implants (Halvorson et al, 1994). In an investigation where implants were fixed in areas with higher and lower bone density, it was observed in the results that bone tissue with lower cortical density favoured pull out strength. This loosening of the screws used for fixation occurs progressively and can compromise bone osseointegration (Akay & Ercan, 2020).

In this study, the incident with the plates occurred almost immediately after the intervention, which leads us to believe that this phenomenon was not one of the possible reasons for the failure. The fact that the implant failure occurred within the first 24-72 hours points to problems due to critical stress concentrations exceeding the strength of the material used, together with structural defects in the design itself. It may also be due to mechanical overloading that has occurred due to anatomical placement of the implant (Zeng et al, 2018).
As mentioned, the plates were redesigned for placement on the caudal aspect of the tibia, but not only was the design changed to accommodate this area. The number of holes for screw anchorage was also redesigned, from 4 holes in the first model, two of them prepared for the placement of 2 locked screws, providing greater long-term stability compared to the use of conventional screws. In the second model, 2 holes were designed for the placement of conventional screws, reducing the anchorage by 50%.

This reduced the structural redundancy of the plate and increased the working lengt. Trials have shown that by increasing the working length, screws closer to the focus deformed by 68-99%, increasing the flexibility of the plate, and the shorter the working length, the more the risk of implant fatigue was reduced (MacLeod et al, 2016). This may have been one of the factors that led to the implant incident.

However, not all subjects in whom the redesigned plates were used experienced such an event. One of the patients evolved favourably, being able to assess the evolution of a patient with the first model of the scaffold implants, designed for the lateral aspect of the tibia, and a patient with the second model of the scaffold, designed for the caudal aspect of the tibia.

The results obtained in the radiological review three months after surgery showed favourable results, evidencing adequate bone regeneration. It should be mentioned that the patient in whom the second m o d e l was used, although he evolved, also presented a slower progression both clinically and radiologically when compared to the first model.

The patient in which the second model was used, although he evolved, also presented a slower progression both clinically and radiologically compared to the other study subject.

This may be due to the sum of the factors mentioned above, such as the lower bone density at the implant fixation face and the reduction of this fixation system. These variables may have resulted in micromovement, which between 10-30% stimulates bone callus formation, but above this percentage, bone neoformation is limited (Cheal et al, 1991).

Although our results after three months of surgery showed bone tissue formation, no osseointegration was observed radiologically. In recent years, scaffold osseointegration has been recurrently studied to solve the problems associated with segmental bone defects, showing that in the first three months the formation of bone callus is seen in the periphery of the implant, at six months more bone growth is observed along the implant, and finally after nine months the filling of the internal space, bone osseointegration, can be seen (Yongfeng Li et al., 2023).

Therefore, we know that the clinical and radiological follow-up time of the animals has been short. Therefore, the next step to verify the formation of bone bridges with the graft is to perform a histological study of the cadavers. In which trabecular bone should be found developing around the scaffold. In addition to the formation of lamellar bone with osteons and Havers canals within the scaffold. The tissue surrounding the implant should show active osteoclasts and osteoblasts, indispensable for the formation of bone tissue. It will be important to find vascularised connective tissue, essential for the nutritional supply necessary for this process (Valamvanos et al, 2024).

Other important findings would be to observe Type I collagen fibres in the samples, essential for the subsequent mineralisation of the osteoid matrix that is in direct contact with the scaffold, which precedes the formation of mature bone. In addition, finding hydroxyapatite crystals between the scaffold and the new bone tissue will be key to knowing that effective union is taking place. Along with this, the absence of an inflammatory response will be crucial, as this indicates that there is no graft rejection. It would be positive to observe that the scaffold begins to degrade into small particles together with multinucleated cells, indicating that the material is being resorbed in a controlled manner (Crovace et al, 2020).

Finally, immunohistochemical analysis can detect osteopontin in t h e newly formed bone, confirming active and adequate osteogenic activity. An increase in alkaline phosphatase would be expected, as this is a marker of osteoblastic differentiation, and is a good indication (Valamvanos et al, 2024).

In summary, what we are looking for histologically after 3 months of evolution are indications that bone regeneration is going well, that there is adequate vascularisation, the absence of inflammatory response and indications that the scaffold is being integrated (Yongfeng Li et al., 2024).

7. CONCLUSION

1. In our study, both scaffold designs were effective in filling the bone defect created and promoted the formation of cortical bone bridging, as evidenced radiologically.

2. We were unable to determine, at least on X-rays, the formation of trabecular bone within the porous implant due to the short time between surgery and euthanasia.

3. The second scaffold model, which we intended to be non-load-bearing, proved to be unstable and insufficient to support the weight of the animals.

4. The scaffold plate needs to be redesigned to avoid further failures and to complete the study.

8. BIBLIOGRAPHY

Haam, D.-W., Bae, C.-S., Kim, J.-M., Hann, S.-Y., Yim, C.-M. R., Moon, H.-S., & Oh, D. S. (2024). Reconstruction of segmental bone defect in canine tibia model utilizing bi-phasic scaffold: Pilot study. International Journal of Molecular Sciences, 25(9), Article 4604.
https://doi.org/10.3390/ijms25094604

Pérez, M. Á., García-Aznar, J. M., & Doblare, M. (2019). Computational simulation of bone tissue regeneration within a specific type of scaffold architecture. Medical Engineering & Physics, 72, 17-26.
https://doi.org/10.1016/j.medengphy.2019.07.00

Aerssens, J., Boonen, S., Lowet, G., & Dequeker, J. (1998). Interspecies differences in bone composition, density, and quality: Potential implications for in vivo bone research. Endocrinology, 139(2), 663–670.

Liu, Y., & Ma, L. (2010). Segmental bone defects: From cellular and molecular pathways to the development of novel biological treatments. *Journal of Cellular and Molecular Medicine, 14*(12), 2657–2674. https://doi.org/10.1111/j.1582-4934.2010.01062.x

Vidal, L., Kampleitner, C., Krissian, S., Brennan, M. Á., Hoffmann, O., Raymond, Y., Maazouz, Y., Ginebra, M. P., Rosset, P., & Layrolle, P. (2020). Regeneration of segmental defects in metatarsus of sheep with vascularized and customized 3D-printed calcium phosphate scaffolds. Scientific Reports, 10(1), 7068. https://doi.org/10.1038/s41598-020-63742-w

Ting D, Petersen SW, Déjardin LM. Bone transport osteogenesis for treatment of canine osteomyelitis. A report of two cases. Vet Comp Orthop Traumatol. 2010;23(2):134-40. doi: 10.3415/VCOT-09-04-0049.

Aldlyami, E., Abudu, A., Grimer, R. J., Carter, S. R., & Tillman, R. M. (2005). Endoprosthetic replacement of diaphyseal bone defects: Long-term results. *International Orthopaedics*, *29*(1), 25-29. https://doi.org/10.1007/s00264-004-0614-6

Vlasa, A., Bud, E., Lazăr, L., Ilies, S., Stoica, A. M., Lazăr, A.-P., Martu, I., & Bud, A. (2025). Systematic Review Regarding the Clinical Implications of Allograft and Alloplastic Bone Substituents Used for Periodontal Regenerative Therapy. *Journal of Clinical Medicine*, *14*(3), 894. https://doi.org/10.3390/jcm14030894

Apard, T., Bigorre, N., Cronier, P., Duteille, F., Bizot, P., & Masquelet, A. C. (2010). Two-stage reconstruction of post-traumatic segmental tibia bone loss with nailing. *Orthopaedics & Traumatology: Surgery & Research*, *96*(5), 549-553. https://doi.org/10.1016/j.otsr.2010.02.010

Gatto, M. L., Groppo, R., Bloise, N., Fassina, L., Visai, L., Galati, M., Iuliano, L., & Mengucci, P. (2021). Topological, Mechanical and Biological Properties of Ti6Al4V Scaffolds for Bone Tissue Regeneration Fabricated with Reused Powders via Electron Beam Melting. *Materials (Basel, Switzerland)*, *14*(1), 224. https://doi.org/10.3390/ma14010224

Gibson, I., Rosen, D., & Stucker, B. (2015). *Additive manufacturing technologies: 3D printing, rapid prototyping, and direct digital manufacturing* (2nd ed.). Springer Science+Business Media.

Gundavda, M., Agarwal, M. G., Puri, A., Gulia, A., & Reddy, K. (2021). Does a modified technique to achieve arthrodesis of the wrist after resection of the distal radius and translocating the ipsilateral ulna as a vascularized graft to reconstruct the defect improve grip strength and outcomes scores? *Clinical Orthopaedics and Related Research*, *479*(7), 1604-1612. https://doi.org/10.1097/CORR.0000000000001604

Sisson, S., Grossman, J. D., & Getty, R. (2001). *Anatomía de los animales domésticos. Tomo I* (5ª ed.). Elsevier Masson.

Pérez Sánchez, L., Silva Torres, M. E., Maldonado Frías, S., Correa-Prado, R., Villarreal-Ramírez, E., Marichi Rodríguez, F., & Serrano-Bello, J. (2025). Andamios impresos en 3D con poros heterogéneos como estrategia de regeneración ósea in vivo. Mundo Nano. Revista Interdisciplinaria En Nanociencias Y Nanotecnología, 18(34), e69828. https://doi.org/10.22201/ceiich.24485691e.2025.34.69828

Velasco Peña, M. A., Toro Toro, L. F., & Garzón-Alvarado, D. A. (2023). Andamios porosos para ingeniería de tejidos óseos: características geométricas, requerimientos de uso, y materiales y métodos de manufactura aditiva. Ingeniería Y Competitividad, 25(3), e–30212572. https://doi.org/10.25100/iyc.v25i3.12572

Li, Y., Liu, H., Wang, C., Yan, R., Xiang, L., Mu, X., Zheng, L., Liu, C., & Hu, M. (2023). 3D printing titanium grid scaffold facilitates osteogenesis in mandibular segmental defects. *NPJ Regenerative medicine*, *8*(1), 38. https://doi.org/10.1038/s41536-023-00308-0

Wieding, J., Lindner, T., Bergschmidt, P., & Bader, R. (2015). Biomechanical stability of novel mechanically adapted open-porous titanium scaffolds in metatarsal bone defects of sheep. *Biomaterials*, *46*, 35–47. https://doi.org/10.1016/j.biomaterials.2014.12.010

Crovace, A. M., Lacitignola, L., Forleo, D. M., Staffieri, F., Francioso, E., Di Meo, A., Becerra, J., Crovace, A., & Santos-Ruiz, L. (2020). 3D Biomimetic Porous Titanium (Ti_6Al_4V ELI) Scaffolds for Large Bone Critical Defect Reconstruction: An Experimental Study in Sheep. *Animals : an open access journal from MDPI*, *10*(8), 1389. https://doi.org/10.3390/ani10081389

Brzezinski, A., Ghodbane, S. A., Patel, J. M., Perry, B. A., Gatt, C. J., & Dunn, M. G. (2017). * The Ovine Model for Meniscus Tissue Engineering: Considerations of Anatomy, Function, Implantation, and Evaluation. *Tissue engineering. Part C, Methods*, *23*(12), 829–841. https://doi.org/10.1089/ten.TEC.2017.0192

Gautier, E., Perren, S. M., & Cordey, J. (2000). Strain distribution in plated and unplated sheep tibia an in vivo experiment. *Injury*, *31 Suppl 3*, C37–C44. https://doi.org/10.1016/s0020-1383(00)80030-3

Chen, H. W., & Luo, C. F. (2015). Extended anterolateral approach for treatment of posterolateral tibial plateau fractures improves operative procedure and patient prognosis. *International journal of clinical and experimental medicine*, *8*(8), 13708–13715. Extended anterolateral approach for treatment of posterolateral tibial plateau fractures improves operative procedure and patient prognosis - PMC

Rubert, M, & De la Piedra, C. (2020). La osteocalcina: de marcador de formación ósea a hormona; y el hueso, un órgano endocrino. *Revista de Osteoporosis y Metabolismo Mineral*, *12*(4), 146-151. Epub 05 de abril de 2021.https://dx.doi.org/10.4321/s1889-836x2020000400007

Pérez Sánchez, L., Silva Torres, M. E., Maldonado Frías, S., Correa-Prado, R., Villarreal-Ramírez, E., Marichi Rodríguez, F., & Serrano-Bello, J. (2025). Andamios impresos en 3D con poros heterogéneos como estrategia de regeneración ósea *in vivo*. *Mundo Nano. Revista Interdisciplinaria En Nanociencias Y Nanotecnología*, *18*(34), e69828. https://doi.org/10.22201/ceiich.24485691e.2025.34.69828

Wieding, J., Jonitz, A., & Bader, R. (2015). Biomechanical stability of novel mechanically adapted open-porous titanium scaffolds in metatarsal bone defects of sheep. *Biomaterials*, *46*, 35-47. https://doi.org/10.1016/j.biomaterials.2014.12.010

Cheal, E. J., Mansmann, K. A., DiGioia, A. M., Hayes, W. C., & Perren, S. M. (1991). Role of interfragmentary strain in fracture healing: ovine model of a healing osteotomy. Journal of Orthopaedic Research, 9(1), 131–14 DOI: 10.1002/jor.1100090116

Schell, H., Thompson, M. S., Bail, H. J., Hoffmann, J. E., Schill, A., Duda, G. N., & Lienau, J. (2008). Mechanical induction of critically delayed bone healing in sheep: radiological and biomechanical results. Journal of Biomechanics, 41(14), 3066–3072. DOI: 10.1016/j.jbiomech.2008.06.038

Akay, G., Ercan, E., & Çağırıcı, T. (2020). Effect of cortical bone thickness and density on pullout strength of mini-implants: An experimental study. American Journal of Orthodontics and Dentofacial Orthopedics, 157(1),8-15. https://doi.org/10.1016/j.ajodo.2019.06.019

Patel, R., Sinha, S., Arya, R. K., Mohaniya, D., Manjhi, B., & Rastogi, A. (2021). Mid-term comparative analysis of orthogonal versus parallel plating in bicondylar tibial plateau fracture. *Journal of Bone and Joint Diseases, 36*(3), 69–73. https://doi.org/10.4103/jbjd.jbjd_11_21

Halvorson, T. L., Kelley, L. A., Thomas, K. A., Whitecloud, T. S., & Cook, S. D. (1994). Effects of bone mineral density on pedicle screw fixation. Spine, 19(21), 2415-2420.
https://doi.org/10.1097/00007632-199411000-00008

Zeng, J. H., Liu, S. W., Xiong, L., Qiu, P., Ding, L. H., Xiong, S. L., Li, J. T., Liao, X. G., & Tang, Z. M. (2018). Scaffolds for the repair of bone defects in clinical studies: a systematic review. *Journal of orthopaedic surgery and research*, *13*(1), 33. https://doi.org/10.1186/s13018-018-0724-2

MacLeod, A. R., Pankaj, P., Simpson, A. H. R. W. (2016). Does screw placement and working length affect the performance of locking plates in osteoporotic bone? *Journal of Orthopaedic Research*, *34*(9), 1527–1534.
https://doi.org/10.1002/jor.23193

Huang, W., Yang, S., Shao, J., & Li, Y. P. (2007). Signaling and transcriptional regulation in osteoblast commitment and differentiation. Frontiers in Bioscience, 12, 3068-3092. DOI: 10.2741/2296

Pan, C.-T., Hsu, W.-H., Cheng, Y.-S., Wen, Z.-H., & Chen, W.-F. (2021). A new design of porosity gradient Ti-6Al-4V encapsulated hydroxyapatite dual materials composite scaffold for bone defects. *Micromachines*, *12*(11), 1294. https://doi.org/10.3390/mi12111294

Li, Y., Yuan, X., Wang, H., Liu, M., Zhang, Q., Zhao, J., Huang, L., & Chen, X. (2024). Metal-bone scaffold for accelerated peri-implant endosseous healing. *Frontiers in Bioengineering and Biotechnology, 12*, 1334072. https://doi.org/10.3389/fbioe.2023.1334072

Valamvanos, T. F., Dereka, X., Katifelis, H., Gazouli, M., & Lagopati, N. (2024). Recent Advances in Scaffolds for Guided Bone Regeneration. *Biomimetics (Basel, Switzerland)*, *9*(3), 153.
https://doi.org/10.3390/biomimetics9030153