EFFICACY OF FIBER-REINFORCED GELATIN NANOHYDROXYAPATITE (nHA) IN HEALING OF FRACTURES WITH SEGMENTAL DEFECTS IN DOGS

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(16-MVP-07)

THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Efficacy of fiber-reinforced gelatin-nanohydroxyapatite (nHA) composite in healing of fractures with segmental defects in dogs"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Efficacy of fiber-reinforced gelatin-nanohydroxyapatite (nHA) composite in healing of fractures with segmental defects in dogs"** is a record of research work done independently by **Archana G. (16- MVP-07)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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EXTERNAL EXAMINER

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ARCHANA G.

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INTRODUCTION

Segmental bone defects occur during revision surgeries, resection of bone tumours, removal of infected bone, correction of malformed bones and trauma. Management of fractures with segmental defects are challenging for the orthopaedician as they are difficult to be reconstructed and heal through a complex process. Grafts are to be implanted at the site of such defects for the fracture to heal. Autografts and allografts are presently the widely adopted grafts for treatment of such defects. Harvest of autografts requires a second surgery on the same patient, consumes time and subjects the patient to further pain. Availability of allografts is limited and is associated with the risk of rejection. These limitations led to the advent of hydroxyapatite- based synthetic bone substitutes.

Hydroxyapatite is similar to bone with respect to chemical composition, is highly biocompatible and has very low immunogenicity. Hydroxyapatite-gelatin and hydroxyapatite-collagen composites are the most commonly used composites in bone tissue engineering. Previous studies have proven that hydroxyapatite with gelatin is osteoconductive in small to medium size segmental defects. Despite the advantages provided, hydroxyapatite is extremely brittle and has very low degradation potential. To overcome these limitations an indigenous novel porous bone substitute- fibre reinforced gelatin nanohydroxyapatite (nHA), was developed by incorporating gelatin nano-hydroxyapatite into electrospun microfibrous sheets of poly-L-lactic acid-(PLLA)-polyvinyl alcohol (PVA) at Center for Nanoscience and Molecular Medicine, Amrita University, Cochin. *In-vitro* and *in-vivo* studies of this indigenously developed bone substitute, in rat and rabbit models, proved that the composite has osteoinductive, osteoconductive and osteointegrative properties required for healing of fractures with segmental bone defects. The degradation potential of the fibre-reinforced gelatin nanohydroxyapatite composite was found improved to the level of 60% of the bone turnover. Such a graft can be clinically employed for treating segmental bone defects which may otherwise delay the healing of fracture or result in non-union.

Hence the present study was conducted to evaluate the efficacy of the fibre-reinforced gelatin nanohydroxyapatite composite in healing of fractures with segmental defects in dogs.

Review of literature

2. REVIEW OF LITERATURE

2.1 INCIDENCE OF FRACTURES OF LONG BONES IN DOGS

In a retrospective study, Singh *et al.*, (2017) reported that 69.96% of all fractures studied in various species of animals were in dogs.

2.1.1 Causes of fractures in dogs

Mahajan *et al.*, (2015) in their retrospective study found that the major cause of fractures in small animals is automobile accidents and falling from a height.

2.1.2 Age

Ali (2013) in a retrospective study observed that young animals are more commonly affected by fractures than adults.

2.1.3 Sex

Minar *et al.*, (2013) in their retrospective study reported that out of the 67 cases studied, 36 dogs (54%) were males and 31 dogs (46%) were females.

2.1.4 Bone

Ali (2010) observed that hindlimb fractures were more common than forelimb in dogs, and femur was the most commonly fractured bone followed by tibia and radius/ulna.

2.2 FRACTURE AETIOLOGY

Newton (1985) reported trauma as the most common extrinsic cause of fractures in dogs and severe contraction of muscles as the common intrinsic cause. The author also reported that direct trauma caused comminuted or multiple fracture, while muscle contractions caused avulsion fractures. Any underlying conditions that made the bone weak caused pathologic fractures.

Denny and Butterworth (2000) reported that direct injury caused by road traffic accidents or falls, were the major cause of fractures. The indirect causes of fracture were uncoordinated movement or muscle contraction. They also reported pathologies of bone, like tumors and dietary imbalance, as causes for pathological fractures.

2.3 SYMPTOMS, SIGNS AND DIAGNOSIS OF FRACTURE

Newton (1985) stated that all complete fractures are presented with impairment or loss of function. Abnormal posture or limb positioning could also indicate fracture, and some weight bearing could be noticed in incomplete or impacted fractures. The author further stated that pain is a common finding at the site of fracture and could be the only clinical indicator for incomplete fractures. Crepitus is seen as a cardinal sign in complete fracture. Swelling, hematoma or contusion could be observed at the site of closed fractures, while lacerations would be present in case of open fractures. Orthogonal radiographic views, including the joint above and below the fracture, are recommended for confirmation.

Langley-Hobbs (2003) stated that two orthogonal radiographic views of the fracture should be taken to confirm diagnosis and location of fracture before attempting repair.

DeCamp (2015) reported pain, localized swelling and tenderness, crepitus and loss of function as some of the clinical signs of fracture. The author stated that at least two orthogonal radiographic views are needed for confirmation of fracture.

2.4 CLASSIFICATION OF FRACTURES

Unger *et al.,* (1990) classified fractures of the four major long bones- humerus, radius/ ulna, femur and tibia/ fibula in dogs and cats, by attributing alpha numeric codes to describe the bone involved, the location of fracture and its type. The first number in the alpha numeric code denoted the bone involved: 1- humerus, 2- radius/ulna, 3- femur and 4- tibia/fibula, while the second number denoted the location of the fracture: 1- proximal, 2- diaphyseal and 3- distal. The proximal and distal fragments were further classified as A- extra articular, B- partial articular and C- complete articular, and diaphyseal fractures are classified as A- simple, B- wedge and C- complex.

Based on the anatomical location of the fracture, Denny and Butterworth (2000) classified fractures as proximal, distal and diaphyseal, and further classified the proximal and distal fractures into articular, epiphyseal, physeal and metaphyseal fractures.

2.5 BIOMECHANICS OF LONG BONE FRACTURES

There are two kinds of forces- physiological forces and non-physiological forces, acting on normal bones. The physiological forces include axial forces, internal stresses, torsional and bending forces, while the non-physiological forces are those acting during trauma. Fractures happen when the amount of force exceeds the bone strength. Axial compression of fracture fragments causes shortening of bone, while axial tension results in lateral expansion. Bending forces cause bending and breaking of bones, while torsional forces cause twisting (Hulse and Hyman, 2003).

2.6 TREATMENT OF FRACTURES

2.6.1 Principles of fracture treatment

As first-aid procedure, fractures must be temporarily immobilized by bandages, splints or casts. Following reduction of the fracture and confirming that the blood supply is not compromised, fractures should be immobilized by suitable method of fixation. Stable fractures could be immobilized by external coaptation techniques, while unstable fractures need to be surgically corrected (Denny and Butterworth, 2000).

AO principles of fracture management which are considered vital for fracture repair and optimal healing are- anatomical reduction and fixation, establishment of stability or splintage, preservation of blood supply to fracture fragments and soft tissues along with early mobilization of the limb and patient (Schatzker, 2005)

Venugopalan (2009) stated reduction of the fracture fragments, retention of the fragments in place and immobilization of the affected limb by external and/or internal fixation techniques as the major steps in fracture correction.

2.6.2 Fracture fixation techniques

2.6.2.1 External coaptation techniques for long bones

Denny and Butterworth (2000) stated that external coaptation could be used to provide added support to fractures that are already treated by internal fixation method, to avoid excessive weight bearing that may cause implant failure. The authors also recommended the application of Robert Jones bandage for fractures that are treated surgically, as the bandage acts as a splint, prevents oedema and is very comfortable for the animal to wear.

Simpson *et al.*, (2001) opined that Robert Jones bandage should not be used as main fixation technique when rigid fixation is required as the padding might loosen, affecting stability and thus hindering fracture healing.

DeCamp *et al.*, (2015) stated that coaptation techniques like application of casts and splints are best suited for greenstick fractures. The authors further stated that Robert Jones bandage is useful in short-term immobilization, and also helps decrease or prevent oedema.

2.6.2.2 Internal fixation techniques

Brinker *et al.*, (1998) stated that selection of internal fixation method should depend on the type of fracture to be corrected and extent of associated soft tissue injury.

Fracture diseases like delayed union and non-union are less common in fractures treated with internal fixation methods. Also, less rigid fixation methods are desirable in diaphyseal fractures as it promotes primary healing. In bones that cannot be reconstructed under compression, techniques like buttress plating or interlocking nailing should be adopted (Denny and Butterworth, 2000).

2.6.3 Bone plating

Denny and Butterworth (2000) stated that bone plates provide good stability to the fracture fragments and help the animal regain early and pain-free mobility, when applied properly. The authors recommend anatomical reduction of fracture fragments prior to application of bone plate. The plate could be applied either in compression, neutralization or buttress mode depending on the type of fracture.

Uhthoff *et al.*, (2006) stated that dynamic compression plates provide stable internal fixation and requires less external immobilization allowing early joint mobility. The authors also reported low incidence of malunion in fractures corrected with plate fixation.

2.6.4 Complications associated with bone plating

Olmstead, 1991 reported that wrong size of implants is the major cause of complication associated with fracture repair using bone plates and screws. Improper number of implants or wrong method of application of the implants also leads to failure of the fracture to heal. The author also stated that even the smallest of fracture gaps should be filled before applying the plate to prevent implant failure.

DeCamp, 2006 stated that bone plates may cause local periosteal circulatory disturbances that may lead to osteoporosis of the bone beneath the plate and interfere with bone growth in young animals.

2.7 MECHANISM OF FRACTURE HEALING

Fracture healing occurs either by direct healing/ osteonal reconstruction or indirect healing intermediate callus formation. Direct healing occurs in fractures where the fracture fragments are in contact or are separated by a small gap (<800µm). Indirect healing happens by transformation of fibrous tissue into bone tissue. This type of healing occurs when there is interfragmentary deformity, impaired blood supply or presence of wide fracture gap, and the stages of indirect fracture healing can further be divided into stage of inflammation, formation of soft callus, formation of hard callus and stage of remodelling (Hulse and Hyman, 2003).

Langley-Hobbs (2003) described radiological findings during second intention fracture healing and reported that the stage of resorption, happening 5 to 10 days post-operative, was characterized by loss of sharp edges of the fracture fragments, and attributed to removal of necrotic tissue. Osteoblasts, fibroblasts and chondroblasts formed in the due course further produce osteoid fibrous tissue and cartilage. Callus, during its stage of formation, happening 10 to 12 days post-surgery, becomes visible on the radiograph when enough mineralization occurs making it radio-dense. The callus increases in radiopacity during the healing phase, around 30 days post-surgery, and fills the fracture gap, thus reducing the fracture lines. Around 90 days post-surgery the cortical shadow and continuous medullary cavity becomes visible during the phase of remodelling. The author also described radiographic findings in direct fracture healing and reported slight widening of fracture gap two to four weeks post fracture correction and attributed it to the initial osteoclastic activity happening at the fracture site resulting in a smudgy appearance on the radiograph.

2.7.1 Factors affecting fracture healing

Claes *et al.,* (1999) in their study to understand the mechanical factors affecting fracture healing stated that under enough blood supply, interfragmentary movement seems to be the major factor that influences fracture healing. Rigid fixation techniques reduce interfragmentary movement leading to formation of minimal callus, while flexible fixation causes formation of callus that further assists healing, but unstable fixation leads to non-union of the fracture fragments. The authors reported that smaller fracture gaps healed faster than larger gap sizes.

DeCamp *et al.,* (2015) opined that when all other factors are constant, and the fracture is reduced appropriately, the rate of healing is mostly influenced by the age of the animal.

Griffon *et al.,* (2005) stated that the fixation technique adopted to correct a fracture is an important factor affecting fracture healing. Bridging techniques like interlocking nailing and external skeletal fixation required less healing time. The authors also observed that fractures in younger animals healed faster.

2.7.2 Complications of fracture healing

Hunt *et al.,* (1980) stated that the immediate complications of fracture healing were due to soft tissue injury, while delayed union, mal union and non-union were the complications encountered later.

Dvorak *et al.,* (2000) opined that complications of fracture healing could be minor like slight malalignment and formation of hypertrophic callus, which are acceptable, or major, like delayed union and non-union, severe malalignment, osteomyelitis and implant failure.

Rovesti (2005) stated that animals with non-union fracture are usually lame with less pain compared to delayed union. The author further stated that radiographically there are no signs of healing of fracture in case of non-union, characterized by the presence of a persistent gap, sclerotic fracture ends and obliterated medullary cavity. The author also stated that valgus is characterized by deviation of the bone axis away from the median plane.

2.8 SEGMENTAL BONE DEFECTS AND THEIR MANAGEMENT

DeCoster *et al.,* (2004) reported that segmental defects occur due to loss of bone during surgical debridement, chronic infection that requires bone resection, long-standing non-unions or tumour resection. The authors recommended the removal of contaminated bone segments and segments that are devoid of any soft tissue attachments. The authors suggested the adoption of limb shortening technique, use of non-vascularized autologous cancellous bone graft and vascularized bone transfer for management of post-traumatic segmental defects of bone.

Pneumaticos *et al.,* (2010) stated that certain synthetic materials of the ceramic and polymer classes can promote cellular responses and can be used to treat critical size defects. They also stated that ceramics like hydroxyapatite integrate with the bone with no fibrous tissue formation as they are structurally similar to the bone mineral phase, and also provide conducive environment for the bone proteins to be absorbed resulting in osteoblast adhesion and proliferation. Also, polymers like polylactic and polyglycolic acid are used for the treatment of segmental bone defects. These polymers gradually degrade, as the cells proliferate through the scaffold, and break down leading to distribution of mechanical stress and strain to the new formed tissue. The authors further stated that polymers could be reinforced with ceramics to increase the bioactivity.

Bernabe´ *et al.,* (2012) reviewed that trauma, infection, surgical procedures or any pathologies lead to segmental defects in bones. The authors stated that segmental defects could be managed using grafts or membranes, or both, and many researches have proven the efficiency of xenografts with osteoconductive and osteoinductive properties.

2.9 BONE GRAFTS AND SUBSTITUTES

Damien *et al.,* (1990) opined that ideal bone substitutes should be biocompatible, easily sterilizable, easy to use and cost effective. Also, they should have properties like, osteointegration, osteoinduction and osteoconduction, and provide a lattice for new bone formation.

Hench (1991) classified bioceramics bone grafts used for fracture repair and reconstruction of damaged bones as single crystal (sapphire), polycrystalline (alumina, hydroxyapatite), glass, glass ceramics or composites (polyethylene hydroxyapatite).

Bone grafts were classified as cancellous, cortical and cortico-cancellous bone grafts based on bone type, and as autografts, allografts and xenografts based on their origin by Denny and Butterworth (2000). The authors also stated that autografts and allografts are successfully used in orthopaedic surgeries while xenografts face rejection.

Moore *et al.*., (2001) stated that an ideal bone graft should have characteristics like osteointegration, osteoconduction, osteoinduction and osteogenesis. The authors further stated that autografts satisfy all the requirements, while allografts are osteointegrative, osteoinductive and osteoconductive but do not possess osteogenic properties. They further stated that the synthetic bone substitutes had only osteoconductive and osteointegrative properties. The authors further mentioned that the limitations of autografts are morbidity and chronic pain at the donor site, while the limitations of using allografts are associated complications of non-union and infection.

Laurencin *et al.,* (2006) classified bone graft substitutes into different classes based on their source and composition as allograft-based, cell-based, factor-based, ceramic-based and polymer-based.

A bone substitute is ‘‘a synthetic, inorganic or biologically organic combination which can be inserted for the treatment of a bone defect instead of autogenous or allogenous bone’’ (Schlickewie and Schlickewie, 2007).

2.10 CERAMIC BASED BONE GRAFT SUBSTITUTES

Damien and Parsons (1991) stated that hydroxyapatite is poorly resorbed into bone and considered it as advantageous. The authors stated that it has low antigenicity and infection rates.

Hench (1991) stated that bioceramics could be composed of a single crystal or polycrystalline, glass or glass-ceramics or composites.

Oonishi (1991) concluded that despite all the advantages offered, the major disadvantages with hydroxyapatite were that the material would not stay in place at bleeding points and has low degree of bone formation within the assemblage of the material.

Kikuchi *et al.,* (2001) conducted a study on the self-organization mechanism of hydroxyapatite/collagen composite in segmental defects created in Beagle tibia and concluded that the mechanical properties exhibited by hydroxyapatite were almost the same as normal bone and observed that hydroxyapatite was resorbed by osteoclasts and new bone was formed by the osteoblasts, as if hydroxyapatite was an autologous graft.

Krishnamurithy (2013) reported that hydroxyapatite was the major component of bone and a potential candidate for bone remodelling. He also stated that its osteoconductivity, biocompatibility and biodegradability promoted bone reconstruction.

The most common ceramic based bone grafts are hydroxyapatite or tricalcium phosphate. Hydroxyapatite had higher mechanical strength than tricalcium phosphate (Campana *et al.,* 2014).

2.11 EFFECTS OF GELATIN AND FIBRES IN BONE GRAFTS

2.11.1 Effects on bone remodelling

Chang *et al.,* (2003) opined that gelatin/nanohydroxyapatite composites mimic natural bone.

Hong *et al.,* (2005) following their *in vitro* study in cell cultures conclude that PLLA-HAP composite was suitable for cell adhesion and proliferation, and the degradation pattern of the composite was found favourable for chondrocyte growth.

Kim *et al.,* (2005) concluded from their *in vitro* study that composites made up of hydroxyapatite with gelatin produced enhanced osteogenic response. Also, in their experimental study on nanocomposites consisting of 20% and 40% hydroxyapatite with gelatin the authors observed that there was increased activity of alkaline phosphatase in the cells growing on the nanocomposites.

Li *et al.,* (2014) from their *in vitro* and *in vivo* study in rats concluded that optimum attachment of cells onto the scaffold is required for their spreading and concluded that the incorporation of gelatin into PLGA nanohydroxyapatite composite enhanced cell adhesion and attributed it to the presence of short oligopeptides that increase cell adhesion, proliferation and spreading. Also, the authors stated that the hydrophilic nature of gelatin favoured cell adhesion. The authors further reported an increase in the expression of the osteogenic genes that prove to help in differentiation of bone marrow stromal cells into osteoblasts.

2.11.2 Effects on mechanical properties

Zhang and Ma (2005) in their study on characterization of poly (α-hydroxyl acids)/hydroxyapatite porous composites reported that PLLA/HAP composites exhibited increased mechanical and tensile strength, and impact energy which could be attributed to the grafted-PLLA molecules incorporated into the composite.

Porous scaffolds made of polymers like poly-α-hydroxyl acids have comparatively less mechanical strength but incorporation of synthetic hydroxyapatite into these porous polymer scaffolds increases the mechanical strength (Venkatesan and Kim, 2014).

Shamaz *et al.*, (2015) in their study on the relevance of fibre-reinforced gelatin nano-hydroxyapatite composite as bone substitute concluded that the addition of a fibrous layer to gelatin-nanohydroxyapatite composite increased the mechanical strength of the scaffold.

2.11.3 Effects on biodegradation of the graft

Shamaz *et al.,* (2015) from their study on fibre reinforced gelatin nanohydroxyapatite in rabbit models concluded that the degradation of the composite took place at par with 60% of the new bone formation and stated that the nano particle size of the mineral component of the composite could have helped in rapid degradation of the implant.

Salmasi *et al.*, (2016) reviewed that the degradation time of polymer/nanohydroxyapatite composites depended on the amount of hydroxyapatite in the composite. Increasing the amount of nanohydroxyapatite delayed degradation.

2.11.4 Effects on radiopacity

Chang *et al.,* (2015) reported that the common biodegradable polymers used as implants lack radiopacity owing to the low specific gravity and electron density of these materials and cannot be identified by the commonly used radiographic techniques.

Ciobanu and Ciobanu (2017) stated that composites without any metallic elements have no radiopacity.

2.12 ANIMAL SELECTION IN BIOMATERIAL RESEARCH

Neyt *et al.,* (1998) studied animal models used in musculoskeletal research and reported that 11% of animal models used for musculoskeletal studies are dogs.

Pearce *et al.,* (2007) reviewed that animal species are chosen as models based physiological and pathological similarities with humans, and with respect to surgical tolerance and resistance to infection. Further, they stated that while selecting animals for studies on bone-implant interaction, understanding of composition and microstructure of the bone and bone remodelling are crucial for later extrapolation of the results to human conditions. The authors also reviewed that dogs are suitable models for orthopaedic studies as the human and canine bone are comparable. There are differences in bone loading between dogs and humans owing to the quadrupedal gait of dogs. Also, the microstructure of bones of humans and canines differ with that the adult human bone consisting of secondary osteonal structure while dogs having mostly secondary osteonal structure at the centre with plexiform bone next to the periosteum and endosteum. Despite the differences in microstructure, canine and human bones are highly similar in composition. But the implant related changes that occur in dog may not be as evident in humans because of the lower remodelling rate in humans.

Reichert *et al.,* (2009) reviewed that the bone weight, density and composition of dogs were closest to that of humans although there are differences in the microstructure.

2.13 SURGICAL APPROACHES TO LONG BONES

The surgeon chooses open or closed reduction of the fracture based on the location of the fracture and the complexity of the fracture. It is recommended to treat articular fractures, simple displaced fractures and comminuted fractures by open method while non-displaced or incomplete fractures by closed reduction.

While closed reduction has the advantage of preserving the blood supply and causes less damage to soft tissue, but it is difficult to restore the normal alignment of the bone. Open reduction allows a surgical approach to the fracture site and allows anatomical reduction of the fracture fragments and fixation with implants. Also, a bone graft can be used if needed. A fully reconstructed bone following open reduction provides advantage with sharing the load bearing of the limb during fracture healing process. Approach through normal fascial planes adequately exposes the fracture fragment thereby avoids the disadvantages associated with open reduction, like damage to soft tissue and disruption of blood supply. A modified method of open reduction is the "open-but-do-not-touch" approach where the fracture fragments could be directly visualized but will not be manipulated. (Houlton and Dunning, 2005)

2.13.1 Humerus

Piermattei and Greely (1993) described a cranio-lateral approach for fractures of humerus between the midshaft and condyles.

Mason (2018) stated that humerus can be approached through cranial, lateral or medial surfaces but the neurovascular structures and the muscle bellies interfere with the approach.

2.13.2 Tibia

Schwarz (2005) stated that to approach the tibial diaphysis the animal should be placed in dorsal recumbency and a cranio-medial skin incision is made from the medial tibial condyle proximally to the medial malleolus distally. This incision is preferred over a medial incision when using a plate as the surgical wound will not directly overly a subcutaneously placed implant.

2.13.3 Radius and ulna

Radius and ulna can be approached from both lateral and medial sides. The lateral approach is preferred when fixation is to be applied to the ulna. (Piermattei and Greely, 1993).

The radius can be approached by a craniolateral or craniomedial skin incision. Craniomedial incision is generally preferred to approach the radius of shaft, while a lateral approach is preferred for proximal to mid diaphyseal region (Toombs, 2005).

2.14 POSTOPERATIVE CARE

2.14.1 Antimicrobial therapy

Nicholson *et al.,* (2002) reported that postoperative wound infection in veterinary practice depends on the duration of surgery, perioperative use of appropriate antibiotic, propofol use and when the surgical site was clipped before the surgery. The authors reported an occurrence of infection of clean-contaminated wounds in 5.9% of all the surgeries performed.

Houlton and Dunning (2005) recommended antibiotic administration at least 30 minutes before anaesthetic induction so that peak serum level of the antibiotic is reached at the time of surgery. The authors also recommended perioperative antibiotic during orthopaedic surgeries exceeding 90 minutes and involved metallic implant fixation. The authors opined that pre and perioperative antibiotic administration reduced post-operative surgical site infection which sis reported to manifest in 2.5 to 4.8% of patients undergoing elective orthopaedic surgeries.

2.14.2 Post-operative analgesia

Denuche *et al.,* (2004) reported that postoperative pain causes complications like delayed wound healing and prolonged period of hospitalization. The authors opined that alleviation of post-operative pain should be a crucial part of post-operative care. Opioids and non-steroidal anti-inflammatory drugs are commonly used to manage post-operative pain. Opioids have several side effects which make NSAIDs a better option. The authors observed similar analgesic effects up to 24 hours post-surgery with perioperative use of meloxicam and ketoprofen.

2.14.3 Post-operative Bandaging

Denny and Butterworth (2000) opined that Robert Jones bandage can be used as first aid to immobilize fractures temporarily and on fractures that have been corrected surgically. The authors stated the bandage is well tolerated despite the bulk.

Houlton and Dunning (2005) recommended the application of soft padding or modified Robert Jones bandage, post-operatively, to reduce swelling and improve patient comfort. The authors also stated that post-operative bandaging helps reduce swelling and pain, prevents contamination of the site and provides support to the limb. Bandages are not required when the fracture is in femur, humerus or axial skeleton, but fractures of the distal extremity require bandaging to reduce swelling.

Fossum (2013) stated that bandages, postoperatively help in protecting the wound, topical medication application, compression of soft tissue and selective immobilization of soft tissue and joints.

2.15 ASSESSMENT OF FRACTURE HEALING

2.15.1 Evaluation of Clinical Condition of the Animal

Dugama (2016) stated that a healthy bright animal would elevate the head and ears, turns towards the stimulus or walks away, when it is approached. Increased or decreased response, abnormal posture and gait are considered abnormal demeanour.

2.15.2 Evaluation of physiological parameters

Newton (1985) reported that there was an increase in temperature 24 to 48 hours after fracture, as a response to the breakdown of the hematoma. Also, animals may become anaemic, due to significant haemorrhage, that occurs when the high-pressure medullary arteries are ruptured during fracture of the bone.

Venugoplan (2009) stated that there was 2 to 3 degree increase in temperature in animals with fracture.

Norkus (2018) stated that the normal heart rate in dogs was 100 to 140 bpm in dogs weighing less than 30lbs, 80 to 120 bpm in dogs weighing 30 to 50 lbs and 60 to 80 bpm in dogs weighing more than 60 lbs. More than 160 bpm or less than 60 bpm in animals of any bodyweight was considered abnormal. The authors also reported that the normal respiratory rate is 20 to 40 breaths/min, and anything above 50 breaths/min or laboured breathing was considered abnormal. Normal dogs had a pink mucous membrane and any other colour was abnormal. The capillary refill time in healthy dogs was less than 2 seconds, and the normal temperature was 100 to 102.9°F.

Patil *et al.,* (2017) reported that there were no major alterations in the physiological parameters like heart rate, respiratory rate and rectal temperature in dogs during the healing period of femoral fractures. All the fluctuations were within the normal range and stated that the elevated physiological parameters that were observed during the pre-operative period could be attributed to the stress caused by the fracture on the animal.

2.15.3 Orthopaedic examination

Welsh *et al.,* (1993) compared Numeric Rating Score (NRS) and Visual Analogue Score (VAS) to assess subjective lameness in sheep. The authors did not observe any significant differences in the lameness score between the two observers. Also, the authors reported that NRS followed normal distribution, while VAS did not.

Cook *et al.,* (1999) used a zero to five score scale to assess lameness in dogs that were treated for fracture of lateral portion of humeral condyles. The scoring pattern was 0 - "no observable lameness", 1 - "intermittent, mild weight-bearing lameness with little if any change in gait", 2 - "consistent, mild weight-bearing lameness with little change in gait", 3 - "moderate weight-bearing lameness - obvious lameness with noticeable head bob and change in gait", 4 - "severe weight bearing lameness - toe-touching only" and 5 - "non-weight-bearing".

Bonde *et al.,* (2004) used a visual scoring method to assess lameness in sows and proposed four grades as 1- no signs of weight lameness, 2- stepping frequently while standing, 3- attempts to relieve limb and 4- reluctance to bear weight on limb.

Quinn *et al.,* (2007) reported that Numeric Rating Scale (NRS) and Visual Analogue Scales (VAS) can be used to subjectively assess limb function in lame dogs. These scales were used to assess the severity of lameness in animals. NRS had four or five descriptions to choose from, while VAS had 100mm line with two vertical lines on its ends marking “sound” and “could not be more lame”. The authors opined that VAS delivered better statistical sensitivity than NRS.

Preston (2012) stated that orthopaedic examination should include localization of pain and presence of swelling, instability or altered range of motion. Also, the animal should be observed for any kind of weight shift evident in single limb lameness, muscle atrophy, joint swelling, limb alignment and deformity while standing and range of motion. The gait of the animal should be assessed when the animal is rising from recumbence and while walking and trotting. Limb manipulation should be done with the animal in lateral recumbence.

DeCamp (2015) recommended the exclusion of possibility of neurological lameness while performing orthopaedic examination and suggested conscious proprioception as method to determine lameness related to neurological conditions.

2.15.4 Radiological examination

Sande (1999) gave a timeline of appearance of radiographic signs of fracture healing in secondary healing in a simple fracture in a dog as follows- five to seven days post-trauma the fracture margins appear to lose sharpness and become smudged with the fracture gap increasing in size, by 10 to 17 days the bony callus appears, by 30 days post fracture reduction the fracture line disappears and the opacity of the callus increases on comparison with adjacent bone and by 90 days the bone is remodelled to its original conformation.

Griffon (2005) stated that direct fracture healing is radiographically depicted by gradual disappearance of the fracture line with absence of any external callus. In contact healing, the zone around the fracture loses radiopacity due to progression of cutting cones through the fracture gap and not because of resorption of the fracture ends and the fracture line eventually disappears. The author also stated that this kind of pattern is observed in fractures that are fixed with compression and rigid fixation. In secondary fracture healing observed in fractures corrected by external coaptation or semi-rigid internal fixation techniques the fracture ends are initially resorbed leading to loss of radiopacity and widening of the fracture gap. The periosteal component of the callus forms first followed by the internal callus which is not generally appreciable on radiographs as it is superimposed by the external callus. Calcified callus can be noticed 10 to 12 days after repair. The fracture line disappears at the formation of hard callus and the fracture gap become radiopaque with the radiopacity of the adjacent bone. Remodelling cannot be well appreciated radiographically. Osteoclastic resorption leads to restoration of medullary blood flow, and the external callus is gradually resorbed with the bone returning to its original shape. The author recommended radiographic evaluation every four to six weeks to check for implant stability, bone alignment, any complications, and to assess bone healing.

Pedrotti *et al.,* (2006) reported that radiography was the most important method of evaluation of fracture healing because it is simple, gives continuous information and could be used for iterative interpretations.

Langley and Hobbs (2003) proposed a six ‘A’s for follow up radiographic evaluation for assessment of fracture healing. According to this, apposition (maintenance of contact between the fracture ends), alignment and angulation (any changes in the alignment of the bone and angulation of the joint), apparatus (intactness of the implant used and its position), activity (any signs of bone healing) and architecture (any change in the surrounding soft tissue or increase of decrease in bone density) were to be noted.

Shamaz *et al.,* (2015) studied the relevance of fibre reinforced gelatin nanohydroxyapatite for bone regeneration and in their study the authors observed that the scaffold was radiolucent and could not be distinctly visualized in radiographs.

2.15.5 Haematological Evaluation

2.15.5.1 Erythrocyte count

Benjamin (2001) recommended the use of electronic instruments to count erythrocytes as with these instruments the cells can be counted faster and the variations observed are lesser than those in manual methods.

Khan *et al.,* (2011) reported that haematology can be used to assess the physiology and pathology of animals. In their study the authors observed that the erythrocyte count was affected by age (higher in adults) and body condition (higher in dogs with poor body condition than in dogs with fair or good body condition) but was not affected by sex.

Rosenfield and Dial (2010) reported that the normal level of erythrocyte count in dogs was 5.5 to 8.5 × 106/µL in conventional units.

Patil *et al.,* (2017) in their study on fracture healing in dogs observed that there was significant elevation in the erythrocyte count on the 60^{th} post-operative day, although within normal range.

2.15.5.2 Packed cell volume (PCV)

Rosenfeld and Dial (2010) reported the normal range of packed cell volume in dogs to be 35 to 55%.

Benjamin (2010) stated that haematocrit is determined by centrifuging blood collected in vials containing anticoagulant and centrifuging at high speed to allow the erythrocytes with highest specific gravity to settle down. The author recommended the use of Wintrobe method or microhematocrit method to determine the packed cell volume. The author further stated that PCV increases due to haemoconcentration.

Patil *et al.,* (2017) observed variations in packed cell volume within the normal range during fracture healing in dogs.

2.15.5.3 Haemoglobin

According to Rosenfeld and Dial (2001) the normal level of haemoglobin in dogs is 12 to 18 g/dL.

Singh *et al.,* (2014) observed no significant difference in the haemoglobin levels in dogs treated for fracture by internal fixation methods on 30th, 60th and 90th post-operative days.

2.15.5.4 Leucocyte count

Klassen (1999) published the reference values for haematology in dogs and reported that the normal leucocyte count in dogs was 4,000 to 15,500/µL.

Kaur *et al.,* (2015) reported neutrophilic leucocytosis in dogs treated for long bone fractures and stated that the leucocytosis could be due the release of corticosteroids as a response to stress, pain, anaesthesia and surgery.

Patil *et al.,* (2017) reported leucocytosis on the pre-operative days and observed significant decrease in the values on the 30th, 60th and 90th post-operative days, although the variations were within the normal range. The authors concluded that this decrease in the leucocyte count could have been due to gradual decrease in inflammation.

2.15.5.5 Erythrocyte sedimentation rate (ESR)

Benjamin (2001) proposed various methods to determine ESR and recommended Wintrobe method which uses blood mixed with anticoagulant. The author stated that dogs have moderately rapid ESR corticosteroids reduce ESR. Also, increase in PCV decreases ESR. ESR is more relevant in dogs and an increase in ESR is an indication of tissue injury.

Khan *et al.,* (2011) stated that the normal range of ESR in dogs was 0-6% in one hour.

Singh (2014) observed non-significant increase in ESR rate on the day of presentation in dogs with fractures and attributed it to the systemic changes due to inflammation caused by the fracture.

2.15.6 Biochemical Assessment

2.15.6.1 Serum enzymes

Rosenfeld and Dial (2010) stated that alkaline phosphatases (ALP) are numerous intracellular enzymes that can be detected in the liver, bone, intestine and placenta. The normal levels of the enzyme are 10-150 IU/L. An increase in the level of this enzyme is observed in conditions like bone growth, liver and gall bladder diseases, and hyperadrenocorticism. Also, increased corticosteroid levels cause a release of ALP from the liver.

Komnenou *et al.,* (2005) reported that following fracture there is an acute phase of reactions that include increased serum ALP and phosphorus, and decreased calcium level. Once the callus formation ends the ALP and calcium levels returned to normal. In their study the authors observed that by the 60th post-operative day, dogs with normal healing had ALP within the reference range while dogs with delayed union had higher level of ALP indicating ongoing osteoblastic activity and callus formation. The authors concluded that along with physical and radiologic examination serial ALP measurement can be an ideal to assess fracture healing in dogs.

Belic *et al.,* (2010) stated that there is a balance between the bone formation-resorption process. Osteoblasts help in the formation of bone while osteoclasts cause resorption of bone for which they produce enzymes and acids which are then released into blood and urine and can be measured by various assays. The authors also stated that biochemical measurements have the advantage of being non-invasive, cost less and also do not interfere with bone metabolism and can hence be repeated. The authors observed from their study that the level of BALP was higher in young dogs as they have more bone turnover.

Sousa *et al.,* (2011) stated that bone specific alkaline phosphatase (BALP) is an enzyme that is released by the osteoclasts that are involved in the bone formation process, and this can be considered a bone formation marker. Tartarate resistant acid phosphatase (TRAP) is a bone resorption marker that is formed due to the breakdown of type I collagen during the bone resorption process. The evaluation of serum markers can help assess the real time healing process of the bone. The authors observed that total ALP and BALP were higher during the post-operative period in the fracture healing groups when compared to those with non-union. TRAP was significantly low up to 60th post-operative day and increased significantly after that.

Patil *et al.,* (2017) reported higher level of alkaline phosphatase during the pre-operative days than during the post-operative days and the authors attributed the proliferating osteogenic cells from the damaged periosteum which is a rich source of the enzyme.

2.15.6.2 Serum calcium and phosphorus

Shin *et al.,* (1994) recommended the use of Arsenazo III method for the estimation of total serum calcium level. The authors stated that the method has high stability and has less errors due to icterus, lipemia and haemolysis. Komnenou *et al.,* (2005) stated that during the acute phase of fracture healing there is hypocalcaemia and hyperphosphatemia. Also, as the process of fracture healing continues the calcium and phosphorus levels return to normal once the callus ceases to form.

Paskalev *et al.,* (2005) observed a decrease in calcium level during the first operative week after osteotomy in dogs.

Rosenfield and Dial (2010) stated that the normal level of calcium is 9.2 to 11.2 mg/dL and the normal level of phosphorus is 2.3 to 5.5 mg/dL in dogs.

Materials and methods

3. MATERIALS AND METHODS

3.1 SELECTION OF CASES

Six dogs presented to the Teaching Veterinary Clinical Complex from February 2017 to October 2018 with fractures, malunion and non-unions that required resection of bone, and resulted in segmental defect, formed the subjects of the study. Cases were selected randomly irrespective of their age, breed and sex, and subjected to surgical correction under general anaesthesia.

3.2 SIGNALMENT

Signalment, including breed, age, and sex and body weight of all the animals were recorded on the day of presentation.

3.3 ANAMNESIS

Detailed history along with details pertaining to the cause of lameness, limb(s) affected, depth of trauma inflicted, progression of lameness and previous treatments (if any) were recorded before confirming the diagnosis.

3.4 MAIN ITEMS OF OBSERVATION

Animals with history of trauma, due to automobile accidents and falls, were examined for internal injuries and bleeding. Fractured limbs were immobilized temporarily, with splints and soft bandages, until surgery. The animals were stabilized with fluids, and antibiotics and analgesics were administered pre-operatively.

3.4.1 Clinical Examination

General body condition and health status of the animal were recorded on the day of presentation and at every two weeks interval post-operatively.

3.4.2 Physiological Parameters

Physiological parameters like rectal temperature (°F), respiration (rate/min), heart rate (beats/min),appearance of conjunctival mucous membrane, pulse rate (per minute) and capillary refill time (s) were recorded on the day of presentation, on the day of surgery and at two weeks interval till eight weeks post-operatively.

3.4.3 Orthopaedic Examination

Orthopaedic examination was performed on the day of presentation and every two weeks until eighth week, by observing from a distance, and by physical examination. Animals were observed while standing, and during progression at different paces. Weight bearing, gait and lameness were scored and graded to evaluate fracture healing.

3.4.3.1 Lameness scoring (Annexure I)

Lameness was scored similar to Mills *et al.*, (2002). Individual scores were given to each animal, based on the clinical signs exhibited by the animal. "One" was graded as stands, walks and trots normally, while "Five" was reluctant to rise and walk, intermittent or continuous non-weight-bearing lameness.

3.4.3.2 Grading of weight bearing (Annexure II)

Weight bearing was scored based on the grading system proposed by McCarthy *et al.*, (2007). The animals were given scores from "One" (bearing weight equally on all limbs while standing and walking) to "Five" (non-weight bearing while standing and walking).

3.4.3.3 Physical examination

The affected limb was examined for presence of swelling, wounds or bruises and crepitation at the site of injury.

3.4.4 Radiographical Evaluation (Annexure III)

On the day of presentation, orthogonal radiographic views of the affected bone were taken to assess the type of fracture and extent of damage. The fractures were classified based on the classification given by Unger *et al.,* (1990).

Orthogonal views of the operated limb were taken immediately post operatively and at every two weeks interval post-operatively to assess fracture healing.

Immediate post-operative radiographs were assessed for the four A's – Apposition, Angulation, Alignment and Apparatus.

The follow up radiographs taken every two weeks were assessed for fracture healing based on the six A's- Apposition, Angulation, Alignment, Apparatus, Activity and Architecture. (Langley Hobbs, 2003)

3.4.5 Haematology and Serum Biochemistry

Haematology and serum biochemistry were performed on the day of presentation and every two weeks post-operatively.

3.4.5.1 Collection of blood

8 ml of whole blood was collected from the cephalic vein of each animal. 4 ml was transferred to potassium EDTA vial for haematology analysis. 4 ml was transferred to clot activator vial and centrifuged to separate serum to perform serum biochemical analysis. A smear was made from the blood collected to estimate differential leucocyte count.

3.4.5.2 Estimation of total erythrocyte count, total leucocyte count, haemoglobin concentration and packed cell volume

Total erythrocyte count (x $10^6/\mu L$), total leucocyte count (x $10^3/\mu L$), haemoglobin concentration (g/dL) and packed cell volume (%) were estimated with the help of automatic veterinary haematology analyser[1] (Exigo EOS Vet) (Plate 1).

3.4.5.3 Estimation of differential leucocyte count

Smears prepared from the blood sample were stained with Giemsa's stain and observed under oil immersion to count differential leucocyte count expressed as percentage of cells.

3.4.5.4 Estimation of serum alkaline phosphatase level

Serum alkaline phosphatase was estimated as per the recommendation of Indian Federation of Clinical Chemistry (2005) by kinetic method using Cormay Liquick Cor-ALP diagnostic kit[3] with the help of biochemical semi-automatic analyser (Merck Microlab 300 biochemistry analyser[2]) (Plate 1).

Estimation was done immediately after separation of serum and the level was expressed as International Units per litre (IU/L).

3.4.5.5 Estimation of serum acid phosphatase level

Serum acid phosphatase was estimated as per the recommendation of Indian Federation of Clinical Chemistry (2005) by kinetic method using Accurex – ACP Diagnostic kit[4] with the help of biochemical semi-automatic analyser (Merck Microlab 300 biochemistry analyser[2]) (Plate 1).

Estimation was done immediately after separation of serum and the level was expressed as International Units per litre (IU/L).

3.4.5.5 Estimation of serum calcium

Serum calcium was estimated by Arsenazo III. Colorimetric method using Spinreact- Calcium estimation kit[5] with the help of biochemical semi-automatic analyser (Merk Microlab 300 biochemistry analyser).

Estimation was done immediately after separation of serum and the level was expressed in mg/dL.

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[1] Exigo Eos Vet Veterinary Haematology Autoanalyzer- Boule medical AB, Domnarvsgattan4, Sweden.

[2] Merck Microlab 300 biochemistry semi-auto analyser, Germany.

[3] Accurex- ACP Diagnostic kit- PZ Cormay SA, Wiosenna 22, Poland

[4]Cormay Liquick Cor-ALP Diagnostic kit- PZ Cormay SA, Wiosenna 22, Poland

[5]Cormay Liquick Cor- Calcium Arsenazo Diagnostic kit- PZ Cormay SA, 22 Wiosenna, Poland

[6]Cormay Liquick Cor-Phosphorus Diagnostic kit- PZ Cormay, Rapackiego 19, Poland

3.4.5.5 Estimation of serum phosphorus

Serum phosphorus was estimated by Phosphomolybdate. UV method using BIO-BAS Phosphorous estimation kit[6] with the help of biochemical semi-automatic analyser (Merk Microlab 300 biochemistry analyser).

Estimation was done immediately after separation of serum and the level was expressed in mg/dL.

3.5 Preparation of Patient

The animals selected for surgery were fasted for at least 12 hours prior to the surgery. Young animals were fasted for 4 to 6 hours prior to surgery.

3.5.1 Preparation of Surgical Site

The surgical site was prepared by clipping and shaving the hair and washing with antiseptic solution. The site was then prepared aseptic using isopropyl alcohol for degreasing, followed by povidone iodine (5%) solution.

3.5.2 Anaesthesia

Anaesthetic protocol was chosen according to the general health status of the animal. Young animals aged less than one year were premedicated with meloxicam[7] (0.3 mg/kg), butorphanol[8] (0.2 mg/kg) and acepromazine[9] (0.02 mg/kg) and induced with midazolam[10] (0.2 mg/kg) and ketamine[11] (5 mg/kg). Older animals were premedicated with meloxicam (0.3 mg/kg), butorphanol (0.2 mg/kg) and xylazine[12] (1 mg/kg) and induced with midazolam (0.2 mg/kg) and propofol[13] (3 mg/kg).

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[8]MELONEX® (5mg/ml)- Intas Pharmaceuticals Ltd. Ahmedabad.

[8]BUTODOL®-L (1mg/ml) - Neon Laboratories Ltd., Boisar road, Thane.

[9]ACEPROMAZINE® (10mg/ml) VET ONE

[10]MEZOLAM® (5mg/ml) - Neon Laboratories Ltd. Boisar road, Thane.

[11]ZOKENT (50mg/ml) - AesmiraMiraculus Pharma Pvt. Ltd. Andheri, Mumbai.

[12]XYLODACTM-Vet (20 mg/ml)- Zydus Animal Health, Ahmedabad

[13]TROYPOFOL (10mg/ml) – Troika Pharmaceuticals Ltd, Uttarakhand

Plate 1

EOS Exigo Vet Veterinary haematology auto analyser

Merck Microlab 300 – Serum biochemistry semi- auto analyser

3.6 BIOGRAFT USED IN THE STUDY

Fibre-reinforced gelatin-nanohydroxyapatite (nHA) composite, prepared by incorporating gelatin nano-hydroxyapatite into electrospun microfibrous sheets of poly (L-lactic acid)-(PLLA)-polyvinyl alcohol (PVA) was the biograft used in the study. The biograft was procured from the Centre for Nanoscience and Molecular Medicine, Amrita Institute of Medical Sciences, Cochin. The material was light-weight, hard, less dense, porous, and available as rectangular blocks of different lengths which could be cut as per requirement. The scaffold absorbs body fluids and becomes soft, allowing it to be moulded to the required segmental space of the fractured bone.

3.7 SURGICAL PROCEDURE (Plate 2 and Plate 3)

Fractures in all the cases were reduced by open surgical method. The fracture fragments following reduction were retained using suitable bone plates and screws, and the segmental defect that had resulted during the course of fracture repair was filled with the biograft. The limb was then immobilized in each case.

3.7.1 Case No. I- Closed Comminuted Mid-diaphyseal Fracture of the Left Tibia and Fibula (42-C)

The fracture site was approached through a curvilinear cranio-medial incision extending from medial tibial condyle cranially to medial malleolus caudally. The splinter devoid of any soft tissue attachment was removed from the site. The fracture ends were surgically debrided. The resultant 8mm segmental defect was bridged with an equal sized piece of bone graft substitute, and the graft and fracture fragments were stabilized using a 3.5mm eight-hole dynamic compression plate and corresponding sized cortical screws of adequate length. The overlying muscles and subcutaneous tissue were apposed. Skin incision was apposed by horizontal mattress suture. The limb was immobilized using modified Robert Jones bandage.

3.7.2 Case No. II- Comminuted Mid-diaphyseal Fracture of Right Tibia and Fibula (42-C)

A cranio-medial approach was adopted to reach the fracture site. The chip splinters devoid of any soft tissue attachment and unable to be incorporated properly for alignment were removed. A 2.7mm 8-holedynamic compression plate and corresponding screws of required length were used to stabilize the fracture and retain the graft in position. A segmental gap of 10mm was observed after debriding the fracture ends. The segmental defect observed was filled using fibre-reinforced gelatin nHA. Overlying muscles, subcutaneous tissue and the skin incision was closed. The limb was immobilized by application of modified Robert Jones bandage.

3.7.3 Case No. III- Non-union of fracture of left humeral mid-diaphysis (12-C)

The fracture site was approached through a cranio-lateral incision. Muscle attachments were separated, and the fracture fragments were exposed. The elephant foot callus at the site was removed and the medullary cavity was reamed. A 2.7mm six-hole dynamic compression plate was applied on the caudal aspect of the bone. As the cranial aspect of the distal fragment did not have enough length to hold the plate and corresponding screw, the plate was applied on the caudal aspect where the distal fragment had enough length to accommodate two screws corresponding to the bone plate after necessary contouring. The fracture gap of about 7.8mm was filled with fibre-reinforced gelatin nHA. The muscles, subcutaneous tissue and skin were sutured, and modified Robert Jones bandage was applied to immobilize the limb.

3.7.4 Case No. IV-Transverse-Overriding Fracture of the Right Radius and Ulna (22-A)

A cranio-medial approach was adopted to expose the fracture site. A part of the proximal fracture fragment was nibbled to correct the overriding**.** The fracture ends had to be nibbled for proper alignment. The resultant segmental defect at the fracture site was filled with fibre-reinforced gelatin nHA after application of a2.7mm eight-hole plate to the cranial aspect of the bone. Muscles and skin were sutured, and modified Robert Jones bandage was applied to immobilize the limb.

3.7.5 Case No. V- Transverse Overriding Fracture of the Left Radius and Ulna (22-A)

The fracture site was approached through a cranio-medial approach. The overriding was reduced after nibbling (for alignment) the proximal fracture end and the resultant segmental defect was filled with fibre-reinforced gelatin nHA after application of a 2mm plate to the cranial aspect of the bone. Robert Jones bandage was applied to immobilize the limb.

3.7.6 Case No. VI- Valgus due to Malunion of Distal Diaphyseal fracture of Left Radius (22-C)

The site was exposed by a cranio-medial incision. A wedge osteotomy was performed on the medial aspect to correct the valgus. A greenstick fracture was created on the lateral aspect of the bone. The alignment was corrected and fibre-reinforced gelatin nHA was placed to fill the excess defect at the site. Muscles and skin sutures were applied. A plaster cast was applied to immobilize the limb and retain the angle of the bone.

3.8 POSTOPERATIVE CARE

Oral tablets of Cefalexin[14] at the dose rate of 25 mg/kg body weight was administered twice a day for seven days, meloxicam[15] at the rate of 0.2 mg/kg body weight for 3 days and tramadol[16] at the rate of 5 mg/kg body weight for 5 days were administered post operatively.

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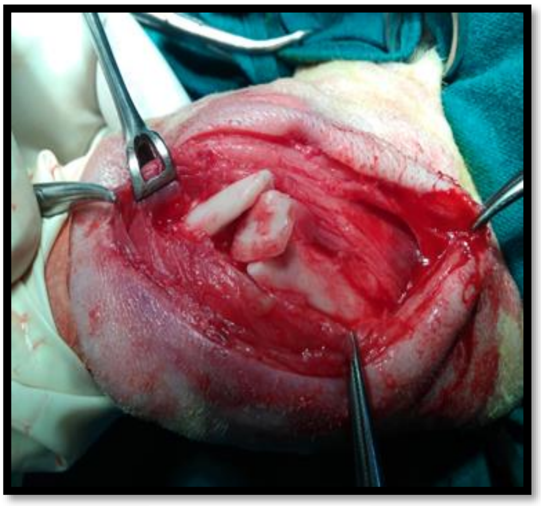
[14]SPORIDEX® 500 mg-Sun Pharmaceuticals, Ind. Ltd. Dewas

[15]METAFLAM® 2.5mg- Savavet, Chennai

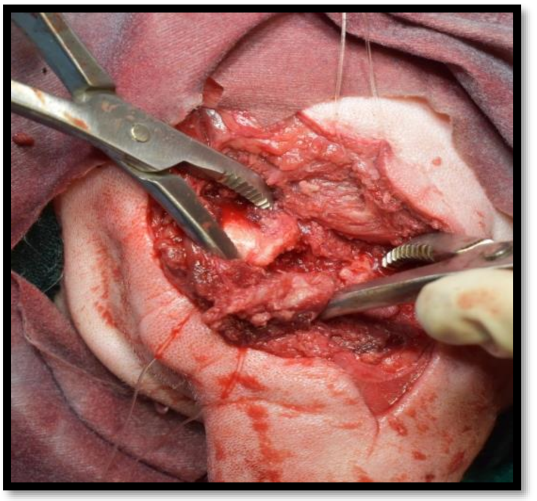
[16]OL_TRAM® 50 mg- HAB Pharmaceuticals. Mumbai

Plate 2

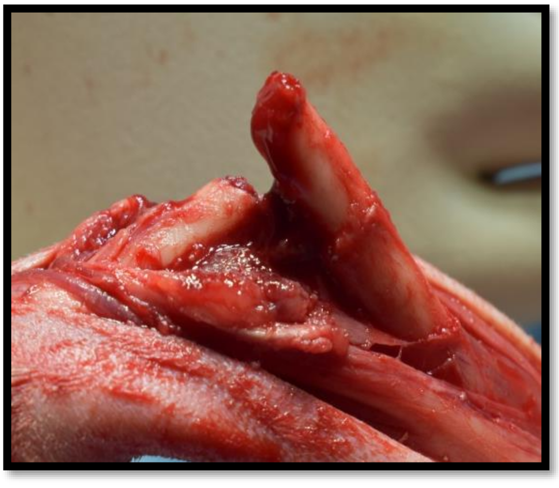
Intraoperative pictures



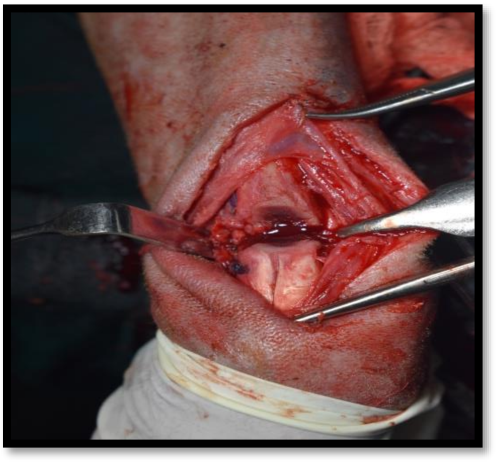
Splinters



Fracture gap after removal of callus



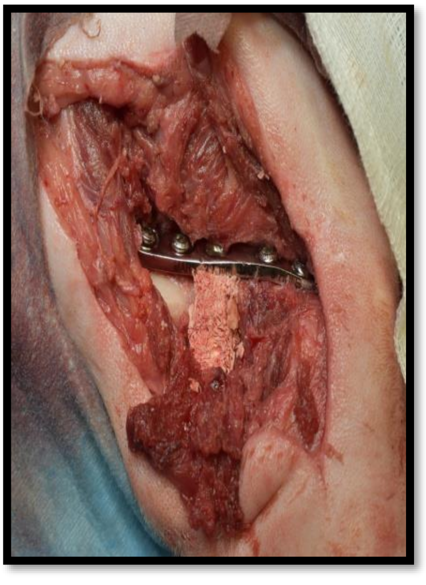
Overriding



Creation of a wedge

Plate 3

Placement of graft in the fracture gap after suitable fixation



Change of bandage, wound dressing and removal of the implant were done depending on the clinical and radiologic findings, and presence or absence of wound site infection.

3.9 STATISTICAL ANALYSIS OF RESULTS

The data recorded was statistically analysed using Latin Square Design. All the tests were done with the help of statistical software IBM SPSS Version 21.

Results

4. RESULTS

The study was carried during the period from August 2017 to November 2018 at the Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Pookode, Kerala.

Six dogs presented to the Teaching Veterinary Clinical Complex from February 2017 to October 2018 with fractures, malunion and non-unions that required resection of bone, and resulted in segmental defect, formed the subjects of the study.

4.1 SIGNALMENT (Table 1)

One of the six dogs was a Labrador, one Pug, one Maltese, one Whippet, one Spitz and one Rottweiler. Of the six dogs three were females and the rest were males. The age of the animals varied from 8 months to thirteen years, and their average body weight was 16kg.

4.2 ANAMNESIS (Table 2)

Five out of the six dogs studied met with road traffic accident, while one had fall from height which resulted in fracture.

Incident happened two months before the day of presentation in one animal (Case No. VI) which was then treated by external coaptation, while in another one (Case No. III) it happened a year before which was then treated with intramedullary pinning and cerclage wiring. One dog (Case No. IV) met with the injury on the day of presentation while two dogs (Case No. II and Case No. V) on the previous day and one (Case No. I) three days prior to presentation.

In Case No. III the fracture failed to heal, and the intramedullary pin was subsequently removed, and the animal was continuing using the affected limb since then.

Table 1. Signalment

Dog No.	**Breed**	**Age**	**Sex**	**Body weight (kg)**
1	Labrador	13 years	Male	30 kg
2	Pug	7 months	Male	10 kg
3	Maltese	8 years	Female	9 kg
4	Whippet	8 months	Female	8 kg
5	Spitz	1.5 years	Female	11 kg
6	Rottweiler	8 months	Male	28 kg

Table 2: Anamnesis

Case No.	**Cause of fracture**	**Day of presentation**	**Previous treatment**
1	Road traffic accident	After 3 days	Nil
2	Road traffic accident	Next day	Nil
3	Fall from a height	After a year	Intramedullary pinning with cerclage wiring
4	Road traffic accident	Same day	Nil
5	Road traffic accident	Next day	Nil
6	Road traffic accident	After 2 months	External coaptation with plaster cast

4.3 PREOPERATIVE EXAMINATION FINDINGS

4.3.1 Clinical Examination

All the animals studied had good body condition and were healthy with normal behaviour. They had no internal injuries or organ damage.

4.3.2 Physiological Parameters

All the animals studied had their temperature, respiratory rate, heart rate, colour of conjunctival mucous membrane and capillary refill time within normal range.

Observations of physiological parameters are shown in Table 3.

4.3.3 Orthopaedic Examination

Four animals were reluctant to walk, while two (Case No. III and Case No. VI) were able to walk. Limping was observed in all animals except the one (Case No. VI) with malunion. One animal (Case No. II) exhibited non-weight bearing lameness on its left hindlimb and two (Case No. IV and Case No. V) on their forelimbs. Partial weight bearing lameness was exhibited on right hindlimb by one animal (Case No. I), while two animals (Case No. III and Case No. VI) had weight bearing lameness on the forelimb, among which one (Case No. III) was lame and the other had angulation of the bone while weight bearing (Case No. VI).

4.3.3.1 Scores for lameness

The scores for lameness was high in four animals (score 4), while it was low in others (score 3 and 2).

Lameness scores obtained by the animals studied is shown in Table No. 4.

4.3.3.2 Grades for Weight Bearing

Grades for weight bearing were high in four animals (grade 4 and 5), while it was low in others (grade 1 and 2).

Grades for weight bearing obtained by the animals studied is shown in Table No. 5.

Table 3. Pre-operative physiological parameters

Parameters	**Case No. 1**	**Case No 2.**	**Case No. 3**	**Case No. 4**	**Case No. 5**	**Case No. 6**
Respiratory rate (/min)	34	36	37	37	35	37
Appearance of conjunctival mucous membrane	Pale pink	Pale pink	Pale pink	Pale pink	Pale pink	Pale pink
Rectal temperature (^{0}F)	102.2	101.9	101.2	101.2	101.3	101.2
Pulse rate (/min)	78	88	85	86	87	88
Heart rate (/min)	82	95	90	98	95	99
Capillary refill time (s)	2	2	2	2	2	2

Table 4. Scoring of lameness (Pre-operative)

Case no.	**Lameness score**
1	4
2	4
3	3
4	4
5	4
6	2

Table 5. Grades for Weight Bearing (Pre-operative)

Case No.	**Before treatment**
1.	4
2.	5
3.	2
4.	5
5.	5
6.	1

4.3.3.3 Physical Examination

Pain and crepitation were observed on manipulation of the fracture in Case No. I, II, IV and V. One animal (Case No. III) exhibited crepitation but had no pain on manipulation. Case No. VI had neither pain nor crepitation. Abnormal mobility was observed in all animals except Case No. VI which displayed valgus deformity of the affected limb.

4.3.4 Radiographical Examination (Plate 4)

Radiographs revealed comminuted fracture of the tibia and fibula in two animals (Case No. I and II), transverse fracture of distal one-third of the diaphysis of both radio-ulna in two animals (Case No. IV and V), hypertrophic callus (elephant foot) of fracture ends- suggestive of non-union fracture, in one animal (Case No. III) and valgus deformity of distal radius and ulna in one animal (Case No. VI). Cerclage wires of previous surgery were observed on the radiographs of Case No. III.

4.3.5 Haematological Evaluation (Table 6)

4.3.5.1 Erythrocyte Count

On day 0, the erythrocyte count was found to be within normal range in all animals. Group mean ± SE on the day of presentation was $5.60 \pm 0.14 \times 10^6$ /µL.

4.3.5.2 Leucocyte Count

The leucocyte count in all animals except Case No. III and Case No. VI were higher but within the normal range.

4.3.5.3 Differential Leucocyte Count

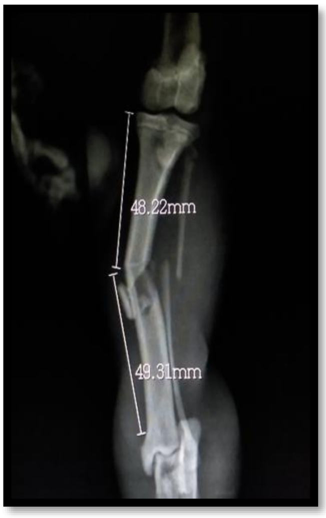
The differential leucocyte count in all animals were within the normal range on the day of presentation.

4.3.5.4 Packed Cell Volume

The packed cell volume in all animals was within the normal range on the day of presentation.

Plate 4

Pre-operative Radiographs



Comminuted fracture of tibia and fibula

Non-union fracture of humerus

Transverse overriding fracture of radius and ulna

Valgus malunion of radius and ulna

4.3.5.5 Haemoglobin Concentration

The packed cell volume in all animals was within the normal range in all animals on the day of presentation.

4.3.5.6 Erythrocyte Sedimentation Rate

The erythrocyte sedimentation rate was within normal range in all animals on the day of presentation.

4.3.6 Serum Biochemistry (Table 7)

4.3.6.1 Serum Alkaline Phosphatase

The serum alkaline phosphatase level was higher but within the normal range on the day of presentation in all animals except one (Case No. III).

4.3.6.2 Serum Acid Phosphatase

The serum alkaline phosphatase level on the day of presentation was higher, but within the normal range, in all animals except one (Case No. III).

4.3.6.3 Serum calcium

The serum calcium level in all animals was within normal range in all animals on the day of presentation.

4.3.6.4 Serum Phosphorus

The serum phosphorus level in all animals was within normal range in all animals.

4.4 POST-OPERATIVE EVALUATION

4.4.1 Clinical examination

All the animals studied had good body condition and were healthy throughout the study period. One animal was hyperactive and was not retaining the bandage.

Table 6. Pre-operative haematological parameters

Parameters	Case No. I	Case No. II	Case No. III	Case No. IV	Case No. V	Case No. VI
Leucocyte count (x 10^3/µL)	13.91	15.42	14.79	13.01	13.24	15.26
Lymphocyte count (%)	15.06	15.12	15.03	16.12	16.34	13.91
Monocyte count (number/µL)	3.00	4.02	4.00	4.04	3.00	4.00
Neutrophils count (%)	80.90	80.86	80.00	78.69	79.16	81.29
Eosinophils (%)	1.02	1.00	0.97	1.00	1.50	1.00
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00
Erythrocyte count (x 10^6/µL)	5.52	5.62	5.53	5.90	5.02	5.98
Haemoglobin concentration (g/dL)	16.60	16.70	16.60	16.90	15.06	17.94
Packed cell volume (%)	49.80	50.10	49.80	53.10	45.18	53.80
Erythrocyte sedimentation rate (mm/min)	7.20	7.50	7.60	7.60	6.80	7.30

Table 7. Pre-operative serum biochemical parameters

Parameters	Case No. I	Case No. II	Case No. III	Case No. IV	Case No. V	Case No. VI
Alkaline phosphatase (IU/L)	109.23	108.92	75.10	108.52	101.24	109.01
Acid phosphatase (IU/L)	1.30	1011	0.64	1.21	1.13	1.10
Calcium (mg/dL)	9.06	9.08	9.05	9.12	9.87	9.12
Phosphorous (mg/dL)	2.60	2.78	2.67	2.12	2.62	3.23

4.4.2 Physiological Parameters

All the physiological parameters recorded varied within the normal range during the entire period of study.

The observations are given in Table 8.

4.4.3 Orthopaedic Examination

4.4.3.1 Lameness Scoring

The scores for lameness in three animals (Case Nos. I, IV and V) decreased during the post-operative period. In two animals (Case No. III and Case No. VI) lameness scores showed a higher value during the 2^{nd} and 4^{th} post-operative week, when compared to that on the day of presentation, which later decreased on 6^{th} and 8^{th} post-operative week. The lameness score decreased during the second post-operative week in one animal (Case No. II) and remained so throughout the study period.

Scores of lameness during post-operative weeks are given in Table 9.

4.4.3.2 Weight Bearing Grade

The grades for weight bearing in three animals (Case No. I, Case No. IV and Case No. V) decreased during the post-operative period. In two animals (Case No. III and Case No. VI) grades for weight bearing showed a higher value during the 2^{nd} post-operative week, when compared to that on the day if presentation, which later decreased on 6^{th} and 8^{th} post-operative week. The grades for weight bearing decreased during the 2^{nd} post-operative week in one animal (Case No. II) and remained so throughout the study period.

Younger animals (Case No. IV, Case No. V and Case No. VI) showed faster regain in weight bearing (Suresh, 2016), except Case No. II.

Grades of weight bearing during 2^{nd}, 4^{th}, 6^{th} and 8^{th} post-operative week are given in Table 10.

Table 8. Post-operative physiological parameters

Parameters	2^{nd} week	4^{th} week	6^{th} week	8^{th} week	p-value
Respiratory rate (/min)	36.50 ± 2.62	36.33 ± 2.60	36.83 ± 1.94	36.83 ± 0.23	0.57^{ns}
Appearance of conjunctival mucous membrane	Pale pink	Pale pink	Pale pink	Pale pink	
Rectal temperature (ºF)	101.53 ± 0.23	101.28 ± 0.08	101.23 ± 0.06	101.37 ± 0.13	0.57^{ns}
Pulse rate (/min)	83.67 ± 1.75	83.17 ± 1.30	82.50 ± 2.05	83.83 ± 1.42	0.64^{ns}
Heart rate (/min)	89.33 ± 2.33	89.00 ± 2.22	88.17 ± 2.61	88.00 ± 2.38	0.12^{ns}
Capillary refill time (s)	2.00 ± 0.00	1.97 ± 0.03	2.00 ± 0.00	2.00 ± 0.00	0.36^{ns}

ns- no significant difference at 0.05 level

Table 9. Post-operative scoring of lameness

Case No	Score Obtained			
	2^{nd} week	4^{th} week	6^{th} week	8^{th} week
1	3	2	2	1
2	3	3	3	3
3	3	2	2	1
4	3	2	1	1
5	3	2	2	1
6	3	2	1	1

Table 10. Post-operative grades for Weight Bearing

Case No	Score Obtained			
	2^{nd} week	4^{th} week	6^{th} week	8^{th} week
1	3	2	1	1
2	3	3	3	3
3	4	2	2	1
4	2	2	1	1
5	2	2	1	1
6	2	2	1	1

4.4.3.3 Physical Examination

One animal (Case No. II) had self mutiliated a few sutures by the third post-operative day. The animal evinced pain on manipulation of the surgical wound site. The site was tender and warm until the end of 2nd post-operative week.

The surgical site was dry and free of any exudates in all other cases by the end of second post-operative week. No swelling, pain or tenderness was observed at the site.

4.4.4 Radiographical Examination

4.4.4.1 Immediate Post-operative Radiograph (Plate 5)

The biograft substituted was radiolucent and was not visible on immediate post-operative radiographs. Good apposition and alignment were observed in all cases. The plates and screws used to fix the fractures and hold the biograft in between the fracture segments were of optimum length and were intact in all cases.

The observations are given in Table 11.

4.4.4.2 Follow-up Radiographic Findings (Plate 6 and Plate 7)

Case No. I: The radiopacity of the biograft in the segmental defect had increased the end of 2nd post-operative week and was detectable on the radiographs. Periosteal activity was observed on the 2nd post-operative week and was observed till the end of 4th post-operative week. Callus was not observed during the period of observation. The biograft was well incorporated into the bone by the end of 8th post-operative week and had radiopacity almost like that of the host bone. Good apposition was observed between the fracture ends and the biograft throughout the observation period.

Case No. II: Two cortical screws in the distal fragment had loosened by the end of 2nd post-operative week. Two more screws in the proximal fragment had loosened by the end of 4th post-operative week, and the biograft appeared to have been getting incorporated into the distal fragment. At the end of 4th post-operative week, there were osteolytic tracts observed around two proximal and three distal screws. At the end of the 8th post-operative week, two of the distal screws were completely loose, causing implant failure. Also, the fracture gap was still evident

Plate 5

Immediate post-operative radiographs

at the end of the study period, although the biograft seemed to have been incorporated into the distal fragment. There were no radiographic signs of biologic activity at the fracture site at the end of 8^{th} post-operative week.

Case No. III: The fracture gap increased at the end of 2^{nd} post-operative week and was then bridged by the end of the study period. At the end of 2^{nd} post-operative week the radiopacity of the biograft had increased. Periosteal and endosteal callus was observed from 4^{th} post-operative week, and the biograft had more radiopacity. Endosteal callus was present at the end of 6^{th} post-operative week through the 8^{th} post-operative week. The graft and the fracture ends had good apposition throughout the study. Signs of remodelling were observed at the end of the study. The radiopacity of the graft had significantly increased by the end of the study period and was moderately incorporated into the bone by the end of 8^{th} post-operative week. No alterations in the alignment or angulation of the bone or joints were observed during the study. The implants were intact and held the fracture ends and the biograft in place throughout the study period.

Case No. IV: At the end of 2^{nd} post-operative week, the radiopacity of the biograft had slightly increased. Mineralization of the fracture gap was observed from the end of 4^{th} post-week. The fracture gap was successfully bridged by new bone formed through the graft, by the end of 8^{th} post-operative week. The biograft was well incorporated into the bone and had the radiopacity of the native bone by the end of the study. The fracture line had disappeared, and the bone resembled the normal bone in architecture and appearance by the end of the study. No callus formation was observed. Good apposition was observed between the fracture ends and the biograft throughout the study period. The implants were stable and maintained rigid fixation throughout the study.

Case No. V: The biograft gained radiopacity at the end of 2^{nd} post-operative week and was visible on radiographs. Mineralization of the segmental defect had started by the end of 4^{th} post-operative week, through the 6^{th} post-operative week. Also, the radiopacity of the biograft increased accordingly. The fracture gap was successfully bridged at the end of 8^{th} post-operative week. The radiopacity of the biograft was almost similar to the host bone by the end of the study. No callus formation was observed. The alignment and apposition of the fracture ends and the biograft was good throughout the study period. The bone plate and screws used were intact.

Case No. VI: There was increase in the radiopacity of the biograft at the end of 2nd post-operative week. The biograft was well incorporated into the segmental defect by the end of 6th post-operative week. The callus formed was in the stage of resorption at the end of 6th post-operative week. Periosteal activity was observed on the 8th post-operative week. The radiopacity of the bone was almost similar to that of the bone at the end of the study period.

The observations of post-operative radiographs are given in Table 12.

4.4.5 Haematological Evaluation (Table 13)

4.4.5.1 Erythrocyte Count

No significant difference was observed in the erythrocyte count during the post-operative observation period. All the observations were within the normal range.

4.4.5.2 Leucocyte Count

No significant difference was observed in the leucocyte count during the post-operative observation period. All the observations were within the normal range.

4.4.5.3 Differential Leucocyte Count

No significant difference was observed in the values of differential leucocyte count during the post-operative period ($p<0.01$). Higher values of neutrophil count, although within normal range were observed during 2nd and 4th post-operative week.

4.4.5.4 Packed Cell Volume

No significant difference was observed in the packed cell volume value during the post-operative observation period. All the values were within the normal range.

Table 11. Immediate post-operative radiographic findings

Case No.	Apposition	Alignment	Angulation	Apparatus
1	1	1	0	1
2	1	1	0	1
3	1	1	0	1
4	1	1	0	1
5	1	1	0	1
6	1	1	1	1

Table 12. Follow-up radiographic findings

Case No.	Apposition				Alignment				Angulation				Apparatus				Activity								Architecture							
																	Endosteal				Periosteal				Periosteal reaction				Osteolysis			
	W2	W4	W6	W8	W2	W4	W6	W8	W2	W4	W6	W8	W2	W4	W6	W8	W2	W4	W6	W8	W2	W4	W6	W8	W2	W4	W6	W8	W2	W4	W6	W8
1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0
2	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	1	1	0	0
3	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0
4	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0
5	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0
6	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0

Plate 6

Radiographs on 2nd post-operative week



Radiographs on 4th post-operative week


Plate 7

Radiographs on 6th post-operative week

Radiographs on 8th post-operative week

4.4.5.5 Haemoglobin Concentration

No significant difference was observed between means of haemoglobin concentration during different observations.

4.4.5.6 Erythrocyte Sedimentation Rate

No significant difference was noticed between observations taken at two weeks interval. Mean erythrocyte sedimentation rate value during each observation was found to be in normal range throughout the period of observation.

4.4.6 Serum Biochemistry (Table 14)

4.4.6.1 Serum Alkaline Phosphatase

There was significant difference ($p<0.05$) in the serum alkaline phosphatase levels during the post-operative period, although the values were within the normal range.

4.4.6.2 Serum Acid Phosphatase

There was significant difference ($p<0.05$) in the serum acid phosphatase level during the post-operative period, although the values were within the normal range.

4.4.6.3 Serum Calcium

There was significant difference in the serum calcium level during the post-operative period, although the values were within the normal range.

4.4.6.4 Serum Phosphorus

There was significant difference ($p<0.05$) in the serum phosphorus levels during the post-operative period, although the values were within the normal range.

4.5 COMPLICATIONS OF FRACTURE HEALING (PLATE 8)

One animal had removed the bandage which led to failure of internal fixation implant.

Table 13. Post-operative haematological parameters

Parameters	2 W	4 W	6 W	8 W	p-value
Leucocyte count (x 10^3/µL)	14.07 ± 0.49	13.75 ± 0.42	12.89 ± 0.53	13.06 ± 0.42	0.07^{ns}
Lymphocyte count (%)	15.12 ± 0.44	15.76 ± 0.56	16.02 ± 0.39	15.63 ± 0.34	0.11^{ns}
Monocyte count (number/µL)	3.46 ± 0.20	4.71 ± 0.36	5.36 ± 0.51	5.31 ± 0.62	0.27^{ns}
Neutrophils count (%)	79.50 ± 0.51	77.50 ± 0.70	77.26 ± 0.43	77.74 ± 0.79	0.69^{ns}
Eosinophils (%)	1.75 ± 0.17	1.43 ± 0.20	1.20 ± 0.16	1.33 ± 0.21	0.13^{ns}
Basophils (%)	0.17 ± 0.17	0.21 ± 0.13	0.00 ± 0.00	0.15 ± 0.15	0.65^{ns}
Erythrocyte count (x 10^6/µL)	5.66 ± 0.12	5.63 ± 0.09	5.66 ± 0.14	5.69 ± 0.12	0.42^{ns}
Haemoglobin concentration (g/dL)	17.0 ± 0.36	16.88 ± 0.27	16.98 ± 0.42	17.07 ± 0.53	0.64^{ns}
Packed cell volume (%)	51.00 ± 1.06	50.63 ± 0.79	50.78 ± 1.21	51.05 ± 0.10	0.68^{ns}
Erythrocyte sedimentation rate (mm/min)	7.03 ± 0.14	7.03 ± 0.10	7.05 ± 0.14	7.05 ± 0.09	0.78^{ns}

ns- no significant difference at 0.05 level

Table 14. Post-operative serum biochemical parameters

Parameters	2^{nd} week	4^{th} week	6^{th} week	8^{th} week	p-value
Alkaline phosphatase (IU/L)	110.20 ± 1.07^{a}	94.44 ± 4.19^{a}	84.46 ± 0.55^{b}	80.70 ± 0.90^{c}	0.003*
Acid phosphatase (IU/L)	1.46 ± 0.04^{a}	1.40 ± 0.08^{b}	1.20 ± 0.10^{c}	0.92 ± 0.08^{d}	0.002*
Calcium (mg/dL)	9.27 ± 0.15^{a}	9.87 ± 0.13^{b}	10.07 ± 0.18^{b}	10.26 ± 0.20^{b}	0.13*
Phosphorous (mg/dL)	3.09 ± 0.12^{a}	3.65 ± 0.12^{b}	3.85 ± 0.14^{b}	3.89 ± 0.29^{b}	0.025*

*- significant difference at 0.05 level

Means having different superscripts are heterogenous

Discussion

5. DISCUSSION

The study was carried during the period from August 2017 to November 2018 on six dogs presented with fractures, malunion and non-unions that required resection of bone, and resulted in segmental defect, formed the subjects of the study. The results of the study are discussed below.

5.1 SIGNALMENT

Four of the animals studied were of age less than one year, and the rest were above one year. Majority of the dogs presented with fracture in this study were young animals and this observation was in accordance with that of Ali (2013). Three of the dogs studied were females while the rest were males. This observation was contrary to the retrospective study report of Minar *et al.,* (2013) where fractures were more common in males than in females. The observations regarding sex of the animal could be due to the small size of the present study group.

5.2 ANAMNESIS

Road traffic accident was the cause of fracture in five of the dogs while fall from a height was the reason in the rest. This observation in the present study was in accordance to that reported by Mahajan *et al.,* (2015). The playfulness and wandering nature of young dogs could have been the reason for road traffic accidents being the common cause of fracture in dogs.

Incident happened two months before the day of presentation in one animal (Case No. VI) which was then treated by external coaptation, while in another one (Case No. III) it happened a year before which was then treated with intramedullary pinning and cerclage wiring. One dog (Case No. IV) met with the injury on the day of presentation while two dogs (Case No. II and Case No. V) on the previous day and one (Case No. I) three days prior to presentation.

In Case No. III the fracture failed to heal, and the intramedullary pin was subsequently removed, and the animal was continuing using the affected limb since then. This could be due to the absence of pain in non-union.

5.3 PREOPERATIVE EXAMINATION FINDINGS

5.3.1 Clinical Examination

All the animals studied had good body condition and were healthy, except for the fracture. They had no internal injuries or organ damage and made them good subjects for surgery under general anaesthesia. Although Denny and Butterworth (2006) stated that animals that had lameness due to trauma had high chances of having internal injuries and multi-organ damage.

5.3.2 Physiological Parameters

Any alterations in the physiological parameters are a reflection on the general health status of the animal. All the animals studied had their physiological parameters within normal range, indicating that they were physiologically stable except for the condition (fracture or malunion or nonunion). Animals that are physiologically unstable should be stabilized prior to the surgery as stated by Denny and Butterworth (2006).

5.3.3 Orthopaedic Examination

Reluctance to walk, limping, partial weight bearing and non-weight bearing lameness were the observations noticed in the animals studied. These symptoms helped in identifying the affected limb. Angulation of the limb on weight bearing was evident in the one with valgus deformity.

Two animals had fractures in the hind limb while four had fractures in the forelimb. This observation was contrary to the observations of Ali (2013) who reported that hindlimb fractures are more common than forelimb fractures. This observation in the study could be due to the small size of the present study group.

5.3.3.1 Scoring of lameness

Lameness scores were high on the day of presentation for all animals except two, one of which (Case No. III) had a non-union fracture and another (Case No. VI) had valgus deformity of the radius and ulna. The lameness scores were directly proportional to the severity of the lameness- higher the score, more severe the lameness and vice versa. The observations were in accordance with the statement of Kaler *et al.*, 2009.

The fracture which had healed but with valgus deformity exhibited least score for lameness.

5.3.3.2 Grading of Weight Bearing

Weight bearing grade was higher in four animals while lower scores were observed in other animals.

The toe-touch weight bearing in one animal with tibia/ fibula fracture could be due to the commencement of weight bearing following three days after injury. Non-weight bearing lameness shown by the other animal with tibia/fibula fracture presented on the next day and two animals with radius/ ulna fracture presented on the same day of injury could be due to the pain inflicted by the injury.

Normal weight bearing with limping observed in the animal with non-union, presented a year later, could be because the animal started to use the limb over a period of time. Rovesti (2005) stated that animals with non-union fractures are usually lame and sometimes show non-weight bearing lameness.

Complete weight bearing observed in the animal with mal-union could be attributed to the healed fracture.

Higher weight bearing grades indicated lesser weight bearing on the affected limb, and vice versa.

5.3.3.3 Physical Examination

Pain and crepitation were the physical examination findings in majority of the cases studied. These findings were in accordance with that reported by DeCamp (2006). Crepitation was observed without pain in one animal while another had neither pain nor crepitation. Abnormal mobility was observed in all except one which had valgus deformity.

Crepitation without pain observed in the one with non-union was in accordance in Rovesti (2005) who stated that pain in non-union fractures are less compared to delayed unions. As the bone remained ununited over a period of one year, the animal may have stopped evincing pain at the site.

Case No. VI had neither pain nor crepitus at the site as the fracture had already healed.

5.3.4 Radiographical Examination

Pre-operative radiographs confirmed diagnosis in all cases.

Non-union in one animal was evident from the presence of elephant foot callus at the fracture site in the radiograph. Valgus malunion was evident from the lateral deviation observed on the radiograph in one case. These observations were similar to those reported by Rovesti (2005).

Pre-operative orthogonal radiographs helped in determining location and type of fracture in the animals studied and helped in choosing the method of fixation. Houlton and Dunning (2005) recommended that at least two orthogonal views be taken prior to surgery to assess the fracture and choose suitable method of fixation.

5.3.5 Haematological Evaluation

All the haematological parameters were within the normal range for all the animals studied.

5.3.6 Serum Biochemistry

5.3.6.1 Serum Alkaline Phosphatase

The level of serum alkaline phosphatase was within the normal range for all animals except one with non-union fracture. This could be attributed to the long-standing condition of non-union in the animal.

5.3.6.2 Serum Acid Phosphatase

The level of serum acid phosphatase was within the normal range for all animals except one with non-union fracture. This could be attributed to the long-standing condition of non-union in the animal.

The mean was 1.08 ± 0.10 IU/L.

5.3.6.3 Serum calcium

Serum calcium level was within the normal range for all animals (9.22 ± 0.13 mg/dL).

5.3.6.4 Serum Phosphorus

Serum phosphorus level was within the normal range for all animals (9.22 ± 0.13 mg/dL).

5.4 POST-OPERATIVE EVALUATION

5.4.1 Clinical examination

All the animals had good body condition and health during the study period. One animal did not retain the Robert Jones bandage applied and is attributed to the hyperactive nature of the animal.

5.4.2 Physiological Parameters

There was no significant difference in the observations on physiological parameters during the post-operative period. This was in accordance with the reports of Patil *et al.*, (2017).

5.4.3 Orthopaedic Examination

5.4.3.1 Lameness Scoring

The scores for lameness in three animals decreased during the post-operative period and could be attributed to alleviation of pain associated with healing of fracture and surrounding tissue, thereby causing reduction in lameness. The lameness scores were directly proportional to the severity of the lameness. In two animals, lameness scores showed a higher value during the 2nd and 4th post-operative weeks, when compared to that on the day of presentation, which later decreased. This could be attributed to the pain inflicted by reaming of medullary cavity in one case and creation of wedge in the other, associated with surgical correction of the respective conditions.

Decrease in lameness score was observed during the second post-operative week in one case and remained so throughout the study period. The fixation failed in this case and the observations on lameness could be attributed to the failure of implant in maintaining fixation.

5.4.3.2 Grading of Weight Bearing

The grades for weight bearing in three animals decreased during the post-operative period. The grades were inversely related to weight bearing. The decrease in the value for the grades could be attributed to the healing of fracture and alleviation of post-operative pain associated with healing of fracture and surrounding tissue, thereby improvement in weight bearing. In two animals, grades for weight bearing showed a higher value during the 2^{nd} post-operative week, when compared to that on the day of presentation. Post-operative pain could have interfered with weight bearing during this time. The grades for weightbearing later decreased, indicating healing of fracture and alleviation of pain allowing return of limb use. The grades for weight bearing decreased during the 2^{nd} post-operative week in one animal and remained so throughout the study period. The internal fixation had become loose in this case which resulted in non-union that lead to altered weight bearing.

Weight bearing was faster in younger animals (Suresh, 2016), except in one case. All the animals regained complete limb function by the end of sixth post-operative week. This could be due to faster bone healing rate in younger animal as reported by Griffon *et al.*, (2005).

5.4.3.3 Physical Examination

One animal had self-mutilated the sutures by the 3^{rd} post-operative day and was not retaining the bandage. This could be attributed to the hyperactive nature of the animal. The animal evinced pain on manipulation of the surgical wound site, which was tender and warm, until the end of second post-operative week, which could be due to the instability at the fracture site caused by implant failure.

No other animals had similar signs indicating healing of the surgical wound.

5.4.4 Radiographical Examination

5.4.4.1 Immediate Post-operative Radiograph

Immediate post-operative radiographs revealed that the biograft used was radiolucent. This observation was in accordance with the observations made by Shamaz *et al.,* (2015) who reported that fibre-reinforced gelatin nano-hydroxyapatite was radiolucent. This radiolucency could be attributed to the absence of any metallic component in the scaffold (Ciobanu *et al.*, 2017).

The fracture ends exhibited good apposition with the biograft, and the implants used were all of optimum size implying that the fracture fixation method adopted was suitable for the specific fractures.

5.4.4.2 Follow-up Radiographic Findings

Case No. I: The radiopacity of the biograft in the segmental defect increased gradually from 2nd post-operative week and was detectable on the radiographs. This could be a result of mineralization of the biograft. Periosteal activity was observed on the 2nd post-operative week and was observed till the end of 4th post-operative week. Callus was not observed during the period of observation which meant that the fixation method was rigid, and there were no micro-movements at the fracture site (Griffon, 2005). The biograft was well incorporated into the bone by the end of 8th post-operative week and had radiopacity almost like that of the native bone. This observation was in accordance with the observations of Shamaz *et al.,* (2015) who reported good osteointegrative property of the biograft. Good apposition was observed between the fracture ends and the biograft throughout the observation period.

Case No. II: Two cortical screws in the distal fragment had loosened by the end of 2nd post-operative week. Two more screws in the proximal fragment had loosened by the end of 4th post-operative week. The animal in this case was hyperactive and overused the limb which could have led to loosening of the screws. This could have been the reason for instability of the fracture fragments. The biograft was found getting incorporated into the distal fragment. At the end of 4th post-operative week, there were osteolytic tracts observed around two proximal and three distal screws which could be due to the movement of the loosened screws. At the end of the 8th post-operative week, two of the distal screws were completely loose, causing implant failure. Also, the fracture gap was still evident at the end of the study period. This could be a sign that the fracture may proceed to be a non-union. There were no radiographic signs of biologic activity at the fracture site at the end of 8th post-operative week, and this observation was in accordance with Denny and Butterworth (2000) who stated the radiographic signs of non-union. The biograft was incorporated into the distal fragment although the fracture gap was still evident.

Case No. III: The fracture gap increased at the end of 2nd post-operative week and was then bridged by the end of the study period which could be due to the activity of osteoclasts (Langley-Hobbs, 2003). At the end of 2nd post-operative week the radiopacity of the biograft had increased which could be due to mineral deposition into the biograft (Langley-Hobbs, 2003). Periosteal and endosteal callus was observed from 4th post-operative week, and the biograft had more radiopacity. Endosteal callus was present at the end of 6th post-operative week through the 8th post-operative week, which is a sign of fracture healing by second intention and could be due to the increased fracture gap during the 2nd post-operative week. The graft and the fracture ends had good apposition throughout the study. Signs of remodelling were observed at the end of the study. The radiopacity of the graft had significantly increased by the end of the study period which implies that there was progressive healing of the bone. The biograft was moderately incorporated into the bone by the end of 8th post-operative week. No alterations in the alignment or angulation of the bone or joints were observed during the study.

Case No. IV: Mineralization of the fracture gap and increase in radiopacity of the biograft was noticed at the end of 2nd post-operative week. New bone formation through the graft which successfully bridged the fracture gap was observed by the end of 8th post-operative week. New bone formation through the biograft and bridging of the fracture gap proves its osteo-conductive property. The biograft was well incorporated into the bone by the end of the study and had radiopacity similar to the native bone by the end of the study. Callus was not noticed during the period of study. Shamaz *et al.,* (2015) reported osteointegrative properties of the biograft in rabbit models. The fracture line had disappeared, and the bone resembled the normal bone in architecture and appearance by the end of the study which indicates complete fracture healing. Good apposition was observed between the fracture ends and the biograft, throughout the study period. The implants were stable and maintained rigid fixation throughout the study.

Case No. V: The biograft gained radiopacity at the end of 2nd post-operative week and was visible on radiographs. Mineralization of the segmental defect had started by the end of 2nd post-operative week, through the 6th post-operative week, which could have been the cause of the increased radiopacity of the biograft. The fracture gap was successfully bridged at the end of 8th post-operative week which indicated that the biograft had osteoconductive property that allowed growth of new bone through the biograft. The radiopacity of the biograft was almost similar to the native bone by the end of the study. Callus formation was not observed during the study period. The alignment and apposition of the fracture ends and the biograft was good throughout the study period which allowed healing of the fracture without any interference. The bone plate and screws used were intact.

Case No. VI: The radiopacity of the biograft placed in the segmental defect increased from the end of 2nd post-operative week. The biograft was well incorporated into the segmental defect by the end of 6th post-operative week. The callus formed was in the stage of resorption at the end of 6th post-operative week. Periosteal activity was observed on the 8th post-operative week. The radiopacity of the healed bone was almost similar to that of the host bone at the end of the study period.

5.4.5 Haematological Evaluation

All the haematological parameters, except neutrophil count, were within the normal range for all the animals, and did not show significant difference during the study period. The higher neutrophil count during the 2nd and 4th post-operative week could be due to the inflammation phase of fracture healing. This observation was in accordance with the observations of Kaur *et al.,* (2015) who reported neutrophilic leucocytosis in dogs treated for long bone fractures

5.4.6 Serum Biochemistry

5.4.6.1 Serum Alkaline Phosphatase

Serum alkaline phosphatase level varied significantly during the post-operative period and was highest during the 2nd post-operative week. This could be attributed to the new bone being formed that increases the serum alkaline phosphatase level.

5.4.6.2 Serum Acid Phosphatase

The serum acid phosphatase level was significantly higher during the 2nd post-operative week and then gradually decreased. This variation the serum acid phosphatase level was in accordance with the observations made by Sousa *et al.,* (2011).

5.4.6.3 Serum Calcium

The serum calcium level varied significantly during the post-operative period. The highest mean for serum calcium level was observed during the end of 8th post-operative week. This observation was in accordance with the reports of Patil *et al.,* (2017) who reported significantly higher serum calcium levels on 60th post-operative day.

5.4.6.4 Serum Phosphorus

The serum phosphorus level varied significantly within the normal range during the post-operative period. This observation was similar to the observations made by Patil *et al.,* (2017) who reported a significant increase in the serum phosphorus level after 60th post-operative day.

Summary

SUMMARY

Segmental bone defects occur when there is loss of a part of the bone. This could result from various causes like resection of bone tumours, removal of infected bone, correction of malformed bone, revision surgeries, *etc*. Treatment of fractures with segmental defects is challenging for the orthopaedician as they are difficult to be reconstructed and heal through a complex process. Correction of segmental bone defects involves the use of bone grafts to fill the defect. Autografts and allografts are the currently used substitutes. Their limitations like the time and pain due to the second surgery performed to obtain autografts, and the risk of rejection with allografts created scope for the development of newer synthetic bone substitutes. *In vitro* studies of such substitutes showed good healing of fractures with segmental bone defects proving that such a graft can be clinically employed for treating segmental bone defects which may otherwise delay the healing of fracture or result in non-union. This formed the basis for the present study where a fibre reinforced ceramic biograft was evaluated for its efficacy to heal segmental bone defects in fractures of dogs.

The study was conducted in six clinical cases of dogs with fractures, malunion and non-unions that required resection of bone during surgical repair and resulted in segmental defects.

Detailed history was collected, clinical signs and physiological parameters were recorded on the day of presentation. Road traffic accident was the most common cause of fracture. Two of the animals were treated earlier for fracture-

one by intramedullary pinning with cerclage wiring and other by external coaptation with plaster cast- which resulted in non-union and malunion, respectively. The animals were subjected to orthopaedic and physical examination. Pre-operative radiographs were taken to assess the bone involved and the type of fracture. Haematology and serum biochemistry were studied on the day of presentation.

Radiographic examination revealed comminuted fracture of tibia and fibula in two animals, transverse overriding fracture of the radius and ulna in two animals, non-union of the humerus in one animal and valgus deformity of the radius and ulna in the other.

All the animals selected for the study were in good health and body condition. Signs of fracture like pain, crepitation and abnormal mobility were observed in all animals except two, which had non-union and malunion. The animal with non-union had crepitation without pain, while the one with malunion had neither pain nor crepitation. Affected limbs were temporarily immobilized by Robert Jones bandage in all cases of fracture.

Under general anaesthesia, standard surgical approaches were adopted for the fracture repair. Bone fragments devoid of any soft tissue attachments were removed from the site in case of comminuted fractures. The fracture fragments were toggled, and ends were nibbled to attain proper alignment in case of transverse overriding fractures. Hypertrophic callus was removed, and the medullary cavity was reamed in case of non-union. Wedge osteotomy was performed, and a greenstick fracture was created align the bone in case of malunion. The resultant segmental defects during fracture repair were filled with fibre-reinforced gelatin nanohydroxyapatite composite after fixing the fractures by suitable internal fixation method. The affected limbs were immobilized by Robert Jones bandage post-operatively. Plaster cast was applied to the affected limb of the animal with malunion after correction.

Immediate post-operative radiographs revealed good apposition between the fracture ends and the biograft in all cases. The biograft appeared radiolucent and the fixation implants used were adequate.

Post-operatively, the animals were evaluated for fracture healing at every two weeks interval from 2nd post-operative week to 8th post-operative week. Clinical, orthopaedic and physical examination was carried out. Radiographical evaluation was performed, physiological parameters were recorded, and haematology and serum biochemistry were studied.

All the animals were in good health during the post-operative period. One animal was hyperactive and did not retain bandage, which led to failure of internal fixation implants. All the physiological parameters in all animals were within the normal range.

Radiographical examination revealed increase in radiopacity of the biograft during the post-operative period in all animals. Periosteal and endosteal callus was formed in one animal. Callus was not observed in any other case. Loosening of screws occurred in one animal which led to implant failure. The fracture gap was successfully bridged in all cases except the one with implant failure. The internal fixation method was adequate and maintained good apposition and alignment in all cases except the one in which the implant failed.

Neutrophil count was higher, although within normal range, during the 2nd and 4th post-operative week which later reduced. All other haematological parameters were within normal range. Serum alkaline phosphatase, serum acid phosphatase, serum calcium and serum phosphorus all varied significantly during the post-operative period but were within the normal range. Case with implant failure had lower levels of alkaline phosphatase and acid phosphatase and was correlated to non-union of fracture.

The only major complication encountered during the study was the implant failure in one case due to the hyperactivity of the animal.

From the present study with six animals with fractures, non-union and mal-union it is concluded that -

- Fibre-reinforced gelatin nanohydroxyapatite is successful in bridging segmental bone defects in fractures
- The biograft has good osteoconductive and osteointegrative properties which helped healing

References

REFERENCES

Ali, L.B.2013. Incidence, occurrence, classification and outcome of small animal fractures: a retrospective study (2005-2010). *World Academy of Science, Engineering and Technology, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*. **7**(3):191-196.

Belić, M., Svetina, A., Kušec, V., Rakočević, S., Grizelj, J., Robić, M. and Turk, R. 2010. Bone alkaline phosphatase, osteocalcin and C-terminal telopeptide as bone turnover markers in canine bitches. *Veterinarski. Arhiv*. **80**(6): 705-713.

Benjamin, M.M. 2001. *Outline of Veterinary Clinical Pathology*. (1st Ed.). Kalyani Publishers, New Delhi, pp: 48-57, 64-67.

Bernabé, P.F.E., Melo, L.G.N., Cintra, L.T.A., Gomes-Filho, J.E., Dezan Jr, E. andNagata, M.J.H. 2012. Bone healing in critical-size defects treated with either bone graft, membrane, or a combination of both materials: a histological and histometric study in rat tibiae. *Clin. Oral Implants Res*. **23**(3): 384-388.

Bonde, M., Rousing, T., Badsberg, J.H. and Sørensen, J.T. 2004. Associations between lying-down behaviour problems and body condition, limb disorders and skin lesions of lactating sows housed in farrowing crates in commercial sow herds. *Livestock Production Science*. **87**(2-3): 179-187.

Brinker WO. 1998. Basic Aspects of Internal Fixation. In: Brinker W. O., Olmstead M. L., SumnerSmith G, Prieur WD. *Manual of internal fixation in small animals. Berlin*: Springer; 8-17.

Campana, V., Milano, G., Pagano, E., Barba, M., Cicione, C., Salonna, G., Lattanzi, W. and Logroscino, G. 2014. Bone substitutes in orthopaedic surgery: from basic science to clinical practice. *J. Mater. Sci. Mater. Med.* **25**(10): 5240-5242.

Chang, M.C., Ko, C.C. and Douglas, W.H. 2003. Preparation of hydroxyapatite gelatin nanocomposite. *Biomaterials*. **24**(17): 2853-2862.

Chang, W.J., Pan, Y.H., Tzeng, J.J., Wu, T.L., Fong, T.H., Feng, S.W. and Huang, H. M., 2015. Development and testing of X-ray imaging-enhanced poly-L-lactide bone screws. *Plo. S. one*, **10**(10): 0140354.

Ciobanu, G. and Ciobanu, O. 2017. Radio-opaque materials based on hydroxyapatite and bismuth. *Rad. Applic.***2**(1): 53-57.

Claes, L., Heitemeyer, U., Krischak, G., Braun, H. and Hierholzer, G.1999. Fixation technique influences osteogenesis of comminuted fractures. *Clin. Orthop. Relat. R.* **365**:221-229.

Cook, J.L., Tomlinson, J.L. and Reed, A.L. 1999. Fluoroscopically guided closed reduction and internal fixation of fractures of the lateral portion of the humeral condyle: prospective clinical study of the technique and results in ten dogs. *Veterinary Surgery*. **28**(5): 315-321.

Damien, C.J. and Parsons, J.R., 1991. Bone graft and bone graft substitutes: a review of current technology and applications. *Journal of Applied Biomaterials*, ***2***(3)**,** pp.187-208.

Damien, C.J., Parsons, J.R., Benedict, J.J. and Weisman, D.S., 1990. Investigation of hydroxyapatite and calcium sulfate composite supplemented with an osteoinductive factor. *Journal of biomedical materials research*. **24**(6): 639-654.

DeCamp, C.E., 2015. *Brinker, Piermattei and Flo's handbook of small animal* *orthopedics and fracture repair*. (4th Ed.). Elsevier Health Sciences. Missouri. pp. 8.

DeCoster, T.A., Gehlert, R.J., Mikola, E.A. and Pirela-Cruz, M.A., 2004. Management of posttraumatic segmental bone defects. *JAAOS-Journal of the American Academy of Orthopaedic Surgeons*, **12**(1): 28-38.

Deneuche AJ, Dufayet C, Goby L, Fayolle P, Desbois C. 2004. Analgesic comparison of meloxicam or ketoprofen for orthopedic surgery in dogs. 2004. **33**(6): 650-60.

Denny, H.R. and Butterworth, S.J. 2000. *A guide to canine and feline orthopaedics.* (4th Ed.). Blackwell Science, Oxford. pp: 18-132.

Duguma A (2016) Practical Manual on Veterinary Clinical Diagnostic Approach. *J. Vet. Sci. Technol.***7**: 337.

Dvořák, M., Nečas, A. and Zatloukal, J. 2000. Complications of long bone fracture healing in dogs: functional and radiological criteria for their assessment. *Acta Veterinaria Brno*. **69**(2):107-114.

Fossum., T. W. 2013.Small animal surgery textbook. 4thedition. Elsevier Health Sciences. St. Louis, Missouri. pp.1050-1052.

Griffon, J. D. 2005. Fracture healing. In: *AO principles of fracture management in the dog and cat*. Georg Thieme Verlag, New York. pp: 72-97.

Hench, L.L. 1991. Bioceramics: from concept to clinic. *J. Am. Ceram. Soc.* **74**(7): 1487-1510.

Hulse, D. and Hyman, B. 2003. Fracture biology and biomechanics. In: Slatter, D. (ed.), *Textbook of small animal surgery*. (3rd Ed.). Saunders, Philadelphia. pp: 1785-1789, 1791-1792.

Hong, Z., Zhang, P., He, C., Qiu, X., Liu, A., Chen, L., Chen, X. and Jing, X. 2005. Nano-composite of poly (L-lactide) and surface grafted hydroxyapatite: mechanical properties and biocompatibility. *Biomaterials*. **26**(32): 6296-6304.

Houlton, J. E. F. and Dunning, D. 2005. Peri-operative patient management. In: *AO principles of fracture management in the dog and cat*. Georg Thieme Verlag, New York. pp: 1-24.

Hunt, J.M., Aitken, M.L., Denny, H.R. and Gibbs, C. 1980. The complications of diaphyseal fractures in dogs: a review of 100 cases. *J. Small Anim. Pract*. **21**(2):103-119.

Kaur, A., Kumar, A., Kumar, D., Mohindroo, J. and Saini, N.S. 2015. Feasibility of C-arm guided closed intramedullary pinning for the stabilization of canine long bone fractures. *Veterinary world*. **8**(12):1410.

Khan, S.A., Epstein, J.H., Olival, K.J., Hassan, M.M., Hossain, M.B., Rahman, K.B.M.A., Elahi, M.F., Mamun, M.A., Haider, N., Yasin, G. and Desmond, J. 2011. Hematology and serum chemistry reference values of stray dogs in Bangladesh. *Open Veterinary Journal*. **1**(1), pp.13-20.

Kikuchi, M., Itoh, S., Ichinose, S., Shinomiya, K. and Tanaka, J. 2001. Self-organization mechanism in a bone-like hydroxyapatite/collagen nanocomposite synthesized in vitro and its biological reaction in vivo. *Biomaterials*. **22**(13): 1705-1711.

Kim, H.W., Song, J.H. and Kim, H.E.2005. Nanofiber generation of gelatin hydroxyapatite biomimetics for guided tissue regeneration. *Advanced functional materials*. **15**(12): 1988-1994.

Klassen. J. K. 2018. Reference values in veterinary medicine. *Laboratory medicine.* **30**(3): 194-197.

Komnenou, A., Karayannopoulou, M., Polizopoulou, Z.S., Constantinidis, T.C. and

Dessiris, A. 2005. Correlation of serum alkaline phosphatase activity with the healing process of long bone fractures in dogs. *Vet. Clin. Pathol*. **34**(1): 35-38.

Krishnamurithy, G. 2013. A review on hydroxyapatite-based scaffolds as a potential bone graft substitute for bone tissue engineering applications. *Journal of Health and Translational Medicine*. **16**(2): 22-27.

Langley-Hobbs, S. 2003. Biology and radiological assessment of fracturehealing. *In Practice*. **25**(1): 26.

Laurencin, C., Khan, Y. and El-Amin, S.F. 2006. Bone graft substitutes. *Expert Rev. Med. Devices.* **3**(1): 49-57.

Li, D., Sun, H., Jiang, L., Zhang, K., Liu, W., Zhu, Y., Fangteng, J., Shi, C., Zhao, L., Sun, H. and Yang, B. 2014. Enhanced biocompatibility of PLGA nanofibers with gelatin/nano-hydroxyapatite bone biomimetics incorporation. *ACS Appl. Mater. Interfaces*. **6**(12): 9402-9410.

Mahajan, T., Ganguly, S. and Para, P. A. 2015. Fracture Management in Animals: A Review. *J. Chem. Bio. Phy. Sci.* **5**(4): 4053-4057.

Mason, D. R. 2018. Humerus fractures. In: Barnhart, M.D. and Maritato, K.C. eds. *Locking Plates in Veterinary Orthopedics*. John Wiley & Sons. pp.107-110.

McCarthy, G., O'Donovan, J., Jones, B., McAllister, H., Seed, M. and Mooney, C. 2007. Randomised double-blind, positive-controlled trial to assess the efficacy of glucosamine/chondroitin sulfate for the treatment of dogs with osteoarthritis. *The Veterinary Journal*. **174**(1):54-61.

Millis, D., Weigel, J. P., Moyers, T. and Buonomo, F., C. 2002. Effect of deracoxib, a
new COX-2 inhibitor, on the prevention of lameness induced by chemical synovitis in dogs. *Vet. Ther.* **3**:453–64.

Minar, M., Hwang, Y., Park, M., Kim, S., Oh, C., Choi, S. and Kim, G. 2013. Retrospective study on fractures in dogs. *Journal of Biomedical Research*, **14**(3):140-144.

Moore, W.R., Graves, S.E. and Bain, G.I. 2001. Synthetic bone graft substitutes. *ANZ J. Surg*. **71**(6): 354-361.

Newton, C. D. 1985. Etiology, Classification and Diagnosis of Fractures. In:C. D. Newton and D. M. Nunamaker (ed.), *Textbook of small animal orthopaedics.* International Veterinary Information Service, Ithaca, New York, USA. pp:1985-185.

Neyt, J.G., Buckwalter, J.A. and Carroll, N.C.1998. Use of animal models in musculoskeletal research. *The Iowa orthopaedic journal*. **18***:* 118.

Nayyer, L., Seifalian, A.M. and Blunn, G.W., 2016.Nanohydroxyapatite
Effect on the Degradation, Osteoconduction and Mechanical Properties of Polymeric Bone Tissue Engineered Scaffolds. *Open Orthop. J.* **10**: 900.

Nicholson, M., Beal, M., Shofer, F. and Brown, D.C. 2002. Epidemiologic evaluation
of postoperative wound infection in clean-contaminated wounds: a retrospective study of 239 dogs and cats. *Vet. Surg*. **31**(6): 577-581.

Norkus, C.L. (ed.,) 2018. *Veterinary technician's manual for small animal emergency*
and critical care. John Wiley & Sons. pp. 123-165.

Olmstead, M.L. 1991. Complications of fractures repaired with plates and screws. *Veterinary Clinics of North America: Small Animal Practice*. **21**(4): 669-686.

Oonishi, H. 1991. Orthopaedic applications of hydroxyapatite. *Biomaterials*. **12**(2): 171-178.

Paskalev, M., Krastev, S. and Filipov, J. 2005. Changes in some serum bone markers
after experimental fracture and intramedullary osteosynthesis in dogs. *Trakia J. Sci*. **3**:46-50.

Patil, M., Dilipkumar, D., Shivaprakash, B.V., Kasaralikar, V.R., Tikare, V.P. and Ramesh, B.K. 2017. Physiological and haemato-biochemical changes during repair of femur fracture in dogs. *The Pharma Innovation*. **6**(8). p.381.

Pearce, A.I., Richards, R.G., Milz, S., Schneider, E. and Pearce, S.G. 2007. Animal models for implant biomaterial research in bone: a review. *Eur Cell Mater*, ***13***(1): 1-10.

Pedrotti, L., Bertani, B. and Mora, R. 2006. Assessment of fracture healing In: Mora.
R. *Nonunion of the Long Bones.* Springer Milan. Verlag Italia. pp: 15-23.

Piermattei, D.L. and Greeley, R.G. 1993. Approach to the Midshaft of the Humerus
Through a Craniolateral Incision**.** In: *An atlas of surgical approaches to the bones of the dog and cat (3rd ed.).* pp:156-159.

Pneumaticos, S.G., Triantafyllopoulos, G.K., Basdra, E.K. and Papavassiliou, A.G. 2010. Segmental bone defects: from cellular and molecular pathways to the development of novel biological treatments. *J. Cell. Mol. Med.* **14**(11): 2561-2569.

Preston. C. 2014. The five-minute orthopedic examination. *The 5th Annual Vet*

Education International Online Veterinary Conference. Australia. July. pp:1-6

Quinn, M.M., Keuler, N.S., Lu, Y.A.N., Faria, M.L., Muir, P. and Markel, M.D. 2007. Evaluation of agreement between numerical rating scales, visual analogue scoring scales, and force plate gait analysis in dogs. *Veterinary Surgery*. **36**(4): 360-367.

Reichert, J.C., Saifzadeh, S., Wullschleger, M.E., Epari, D.R., Schütz, M.A., Duda, G.N., Schell, H., van Griensven, M., Redl, H. and Hutmacher, D.W.2009. The challenge of establishing preclinical models for segmental bone defect research. *Biomaterials*. **30**(12): 2149-2163.

Rosenfeld, A.J. and Dial, S.M. 2010. *Hematology: The Erythrocytes, White Blood Cells, and Immune System.* In*:* Clinical pathology for the veterinary team. John Wiley & Sons. pp: 33-45.

Rovesti, L. G. 2005. Complications of fracture treatment. In: *AO principles of fracture management in the dog and cat*. Georg Thieme Verlag, New York. pp: 394-424.

Salmasi, S., Nayyer, L., Seifalian, A.M. and Blunn, G.W. 2016. Nanohydroxyapatite Effect on the Degradation, Osteoconduction and Mechanical Properties of Polymeric Bone Tissue Engineered Scaffolds. *The open orthopaedics journal*. **10**: 900.

Sande, R., 1999. Radiography of orthopedic trauma and fracture repair. *Veterinary Clinics: Small Animal Practice*. **29**(5): 1247-1260.

Schatzker, J. 2005. Introduction, AO philosophy and principles. In: *AO principles of fracture management in the dog and cat*. Georg Thieme Verlag, New York. pp: xv-xix.

Schlickewie, W. and Schlickewie, C. 2007. The use of bone substitutes in the treatment ofbone defects—the clinical view and history. *Macromolecular Symposia*. **253**:10–23.

Schwarz, G. 2005. Fractures of the proximal tibia. In: *AO principles of fracture management in the dog and cat*. Georg Thieme Verlag, New York. pp: 310-313.

Shamaz, B.H., Anitha, A., Vijayamohan, M., Kuttappan, S., Nair, S. and Nair, M.B. 2015. Relevance of fiber integrated gelatin-nanohydroxyapatite composite scaffold for bone tissue regeneration. *Nanotechnology*. **26**(40): 405101.

Shin, D. H. M. D., Yoon, K. J. M. D., Kwon, S. K. M. T. and Kang, C. G. M. D. 1994. Determination of Total Calcium in Serum with Arsenazo III Method. *Korean J. Clin. Pathol.* **14**(1):12-19

Simpson, A.M., Radlinsky, M.A. and Beale, B.S., 2001. Bandaging in dogs and cats:
external coaptation. *Compend. Contin. Educ.Pract. Vet*. **23**: 157-164.

Singh, G. 2014. C-reactive protein and erythrocyte sedimentation rate: Continuing role for erythrocyte sedimentation rate. *Advances in Biological Chemistry*, **4**(1): 5.

Singh, C.K., Sarma, K.K., Kalita, D., Deuri, B. and Nath, P.J., 2017. Review on Fracture, Dislocation and Neurological Affections in Animals between April 2011 to March 2013 around the Urban and Suburban Area of Guwahati, India. *International Journal of Current Microbiology and Applied Sciences*, *6*, pp.1540-1550.

Sousa, C., Abreu, H., Viegas, C., Azevedo, J., Reis, R., Gomes, M. and Dias, I. 2011.
Total serum and bone alkaline phosphatase and tartrate-resistant acid phosphatase activities for the assessment of bone fracture healing in dogs. *Brazilian Archives of Veterinary Medicine and Animal Science*. **63** (4):1007-1011.

Toombs. 2005. Fractures of proximal the radius. In: *AO principles of fracture* *management in the dog and cat*. Georg Thieme Verlag, New York. pp: 230-236.

Uhthoff, H.K., Poitras, P. and Backman, D.S. 2006. Internal plate fixation of fractures: short history and recent developments. *Journal of orthopaedic science*. **11**(2): 118-126.

Unger, M., Montavon, P.M. and Heim, U.F.A. 1990. Classification of fractures of long bones in the dog and cat: Introduction and clinical application. *Vet. Comp.Orthop.Traumatol.* **3**(2): 41-50.

Venugopalan, A. 2009. *Essentials of veterinary surgery* (8th Ed.). Oxford and IBH Publishing Co., New Delhi, pp: 166-173.

Venkatesan, J. and Kim, S.K. 2014. Nano-hydroxyapatite composite biomaterials for

bone tissue engineering—a review. *J. Biomed. Nanotechnol.* **10**(10): 3124-3140.

Welsh E. M., Gettinby G. and Nolan A. M. 1993. Comparison of a visual analogue scale and a numerical rating scale for assessment of lameness, using sheep as a model. *Am. J. Vet. Res.***54**: 976-983.

Zhang, R. and Ma, P.X.1999. Poly (α-hydroxyl acids)/hydroxyapatite porous composites for bone-tissue engineering. I. Preparation and morphology. *Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials*. **44**(4): 446-455.

EFFICACY OF FIBER-REINFORCED GELATIN NANOHYDROXYAPATITE (nHA) IN HEALING OF FRACTURES WITH SEGMENTAL DEFECTS IN DOGS

ARCHANA G.

(16-MVP-07)

ABSTRACT

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF VETERINARY SCIENCE

(Veterinary Surgery and Radiology)

2019

Faculty of Veterinary and Animal Sciences

Kerala Veterinary and Animal Sciences University

DEPARTMENT OF VETERINARY SURGERY AND RADIOLOGY
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
POOKODE, WAYANAD- 673 576
KERALA, INDIA

ABSTRACT

Segmental bone defects occur when there is loss of a part of the bone. This could result from various causes like resection of bone tumours, removal of infected bone, correction of malformed bone, revision surgeries, *etc*., and are difficult to treat. Correction of segmental bone defects involves the use of bone grafts to fill the defect. Autografts and allografts are the currently used substitutes. Their limitations created scope for the development of newer synthetic bone substitutes. *In vitro* studies of such substitutes showed good healing of fractures with segmental bone defects proving that such a graft can be clinically employed for treating segmental bone defects which may otherwise delay the healing of fracture or result in non-union. This formed the basis for the present study where a fibre reinforced ceramic biograft was evaluated for its efficacy to heal segmental bone defects in fractures of dogs.

The study was conducted in six clinical cases of dogs with fractures, malunion and non-unions that required resection of bone during surgical repair and resulted in segmental defects.

Under general anaesthesia, standard surgical approaches were adopted for the fracture repair and the resultant segmental defect after correction in each case was filled fiber-reinforced gelatin nanohydroxyapatite composite post fixation with suitable internal fixation method. Post-operatively, the animals were evaluated for fracture healing at every two weeks interval from 2nd post-operative week to 8th post-operative week. Clinical, orthopaedic and physical examination was carried out. Radiographical evaluation was performed, physiological parameters were recorded, and haematology and serum biochemistry were studied.

Lameness scores and weight bearing grades improved in all cases except one. Radiographical examination showed progressive healing of fracture throughout the study in all animals except one. The biograft was incorporated into the native bone and the fracture gap was successfully bridged in all cases except the one with implant failure, by the end of the study. The internal fixation method was

adequate and maintained good apposition and alignment in all cases except the one in which the implant failed.

It could be concluded from the study that fiber-reinforced gelatin nanohydroxyapatite composite successfully bridges segmental bone defects and has good osteoconductive and osteointegrative properties which help fracture healing in segmental bone defects.

Appendices

Annexure I- Lameness scoring

Score	Clinical Signs
1	Stands, walks, and trots normally
2	Stands normally, slight lameness under special circumstances or inconsistent lameness when trotting
3	Stands normally, obvious lameness when trotting, consistent lameness when walking
4	Abnormal position while standing, obvious lameness under any circumstances
5	Reluctant to rise and walk, intermittent or continuous non-weight-bearing lameness

Annexure II- Weight bearing score

Score	**Posture and locomotion**
1	Equal on all limbs standing and walking
2	Normal standing; favours affected limb when walking
3	Partial weight-bearing standing and walking
4	Partial weight-bearing standing; non-weight-bearing walking
5	Non-weight-bearing standing and walking

Annexure III- Classification of fractures

Bone	**Symbol**
Humerus	1
Radius and ulna	2
Femur	3
Tibia and fibula	4

Segment	**Symbol**
Proximal	1
Diaphyseal	2
Distal	3

Type of diaphyseal fracture	**Symbol**
Simple	A
Wedged	B
Complex	C

Acad. Form No. 19

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY

Faculty of Veterinary and Animal Sciences

PROGRAMME OF RESEARCH WORK FOR THESIS FOR MASTERS' DEGREE

1. Title of thesis:

Efficacy of fiber-reinforced gelatin-nanohydroxyapatite (nHA) composite in healing of fractures with segmental defects in dogs

2(a) Title of the departmental / KVASU research project of which this forms a part:

Not applicable

2(b) Code No. if any, and order by which the departmental / KVASU research project is approved:

Not applicable

3(a) Name of the student:

Archana G.

3(b) Admission No:

16-MVP-07

4(a) Name of the Major Advisor (Guide)

Dr. Sooryadas S.

4(b) Designation:

Assistant Professor,
Department of Veterinary Surgery and Radiology,
College of Veterinary and Animal Sciences,
Pookode, Lakkidi P.O.
Wayanad, PIN 673 576

5. Objective of the study:

To study the efficacy of fiber-reinforced gelatin nanohydroxyapatite composite in healing of fractures with segmental defects in dogs

6. Practical / Scientific utility:

Healing of fractures with tissue loss is always a challenge to practitioners owing to the specific healing pattern of bones. Such fractures were treated using autografts, allografts and xenografts. The associated complications and the limited availability of such materials have led to the quest for synthetic bone graft substitutes.

Hydroxyapatite has been considered as one of the best biocompatible bone substitute because of its structural similarity to cancellous bone, excellent osteoconductive, osteoinductive and bone-binding properties. But, hydroxyapatite is extremely brittle and degraded very slowly in the body.

In a pursuit to overcome this, a novel biomaterial is developed indigenously at Center for Nanoscience and Molecular Medicine, Amrita University (Fiber reinforced gelatin-nanohydroxyapatite composite scaffold). Preliminary studies using this material demonstrated enhanced mechanical stability and cytocompatibility *in vitro*. Further, the biomaterial could enhance new bone formation at critical sized segmental defect in rat femoral bone when compared to commercially available hydroxyapatite.

The present study is undertaken to evaluate the efficacy of fiber-reinforced gelatin-nanohydroxyapatite composite as a scaffold for the management of fractures of bones with segmental defect in dogs, under clinical conditions.

7. Important publications on which the study is based:

Cooke (1992) observed that despite all the advantages offered, the brittleness and slow degradation when implanted into the body limits the use of hydroxyapatite bioceramics as bone substitutes.

Du *et al.* (1998) studied the resistance of nanohydroxyapatite-collagen composite to localized pressure and concluded that it was isotropic, and microhardness of the composite reached the lower limit of that of the femur *compacta*, in rabbits.

According to Gasser (2000) biomaterial composites as bone implants are of special interest owing to their reduced weight, radiolucency and lower stiffness when compared to metals.

Kikuchi *et al.* (2001) studied hydroxyapatite reinforced with collagen fibers in Beagle tibiae and observed that the composite was incorporated into the remodeling process of bone, resorbed by osteoclastic cells, and new bone was formed by osteoblasts after the resorption, as if the composite were grafted autologous bone.

Porous gelatin-hydroxyapatite composite, used for bone tissue engineering, when subjected to Dynamic Mechanical

Thermal Analysis showed a much higher bending modulus (36-38 GPa) which was quite higher (7-30 GPa) than that of dry cortical bone (Askarzadeh *et al.*, 2004).

Joaquim *et al.* (2008) demonstrated the biocompatibility of hydroxyapatite which was evident from its non-cytotoxic effect over L929 cells. The authors also stated that the rat bone marrow stromal cells adhered, proliferated well and remained viable on the macroporous hydroxyapatite scaffolds.

According to Ballo *et al*. (2009) fiber reinforcement renders biomaterials stronger and more durable, thus making them better bone substitutes in pigs.

Anjana *et al.* (2016) evaluated the osteoinductive and endothelial differentiation potential of platelet rich plasma incorporated gelatin-nanohydroxyapatite fibrous matrix, and concluded that the combination could be a potential candidate for bone tissue engineering applications.

8. Outline of technical programme:

The study will be conducted in dogs presented to the Teaching Veterinary Clinical Complex, Pookode with symptoms suggestive of fracture of bones. The dogs will be subjected to clinical and orthopaedic examination. Orthogonal radiographic views will be taken to confirm fractures. Minimum of six cases of bone fractures with segmental defect will be selected and subjected to surgical management under general anesthesia.

The fractured bone will be approached by standard techniques and the segmental defect will be filled with fiber-reinforced gelatin-nanohydroxyapatite composite provided by Centre for Nanosciences and Molecular Medicine, Amrita Institute of Medical Sciences, Kochi. The fractured fragments will be retained by appropriate fixation technique.

Clinical, hematological and serum biochemical studies will be conducted pre-operatively and at two weeks interval up to eighth week post-surgery, and radiographic evaluation will be done pre-operatively, immediately post-surgery and at two weeks interval up to eighth week post-surgery.

The data will be recorded and statistically analyzed.

9. Main items of observation:

1. Signalment and anamnesis
2. General clinical condition of the animal
3. Physiological parameters-
    i. Rate of respiration
    ii. Heart rate
    iii. Pulse rate
    iv. Rectal temperature
    v. Capillary refill time
4. Orthopedic examination
5. Radiographic evaluation
6. Hematological parameters-
    i. Total leukocyte count
    ii. Differential leukocyte count
    iii. Total erythrocyte count
    iv. Hemoglobin concentration
    v. Volume of packed red cells
    vi. Erythrocyte sedimentation rate
7. Serum biochemical analysis –
    i. Calcium
    ii. Phosphorus
    iii. Alkaline phosphatase
    iv. Acid phosphatase
8. Complications, if any

10. Facilities

(a) Existing

Existing facilities in the Department of Veterinary Surgery and Radiology, and other departments in the College of Veterinary and Animal Sciences, Pookode will be utilized.

(b) Additional facilities required

Implants and serum analysis kits

11. Duration of study

Four semesters

12. Financial estimate

Orthopaedic implants, anesthetics, and antibiotics	Rs. 10,000/-
Biochemical analysis reagents and contingencies	Rs. 8,000/-
Miscellaneous	Rs. 7,000/-
Total	**Rs. 25,000/-**

Signature of the student

Signature of the Major Advisor

Place: Pookode

Date:

Name, Designation and Signature of Members of Advisory Committee

Chairman-

Dr Sooryadas S.,
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APPENDIX-1

References –

Anjana, J., Kuttappan, S., Keyan, K.S. and Nair M.B. 2016. Evaluation of osteoinductive and endothelial differentiation potential of platelet-rich plasma incorporated gelatin-nanohydroxyapatite fibrous matrix. *J Biomed. Mater. Res. B. Appl. Biomater.* **104**: 771-781.

Askarzadeh, K., Orang, F. and Moztarzadeh, F. 2004. Fabrication and characterization of a porous composite scaffold based on gelatin and hydroxyapatite for bone tissue engineering. *Iran. Polym. J.* **14:** 511-520.

Ballo, A. M., Akca, E. A., Ozen, T., Lassila, L., Vallittu, P. K. and Närhi, T. O. 2009. Bone tissue responses to glass fiber-reinforced composite implants – a histomorphometric study. *Clin. Oral. Implants. Res*. **20**: 608−615.

Cooke, F.W. 1992. Ceramics in orthopedic surgery. *Clin. Orthop.* **276**: 135–46.

Du, C., Cui, F. Z., Feng, Q. L., Zhu, X. D. and deGroot, K. 1998. Tissue response to nano-hydroxyapatite/collagen composite implants in marrow cavity. *J. Biomed. Mater. Res.* **42**: 540-548.

Gasser, B. 2000. About composite materials and their use in bone surgery. *Injury.* **31**: 48-53.

Joaquim, M. O., Silva, S. S., Malafaya, P. B., Rodrigues, T. M., Kotobuki, N., Hirose, M., Gomes, E. M., Mano, J. F., Ohgushi, H. and Reis, R. L. 2008. Macroporous hydroxyapatite scaffolds for bone tissue engineering applications: Physicochemical assessment of rat bone marrow stromal cell viability. *J. Biomed. Mater. Res. A.* **91**: 175-186.

Kikuchi, M., Itoh, S., Ichinose, S., Shinomiya, K. and Tanaka, J. 2001. Self-organization mechanism in a bone-like hydroxyapatite/collagen nanocomposite synthesized in vitro and its biological reaction in vivo. *Biomaterials.* **22**: 1705-1711.

APPENDIX II

Time frame of work

Semester 1

1. Collection of literature
2. Planning of the programme for research
3. Preparation of synopsis

Semester 2

1. Collection of literature
2. Starting research in clinical cases

Semester 3

1. Research work continued

Semester 4

2. Continuation of research work
3. Analysis of data
4. Compilation and preparation of thesis
5. Submission of thesis

CERTIFICATE

Certified that the research project has been formulated observing the stipulations laid down under the Prevention of Cruelty to Animals Act (Amendment, 1998).

Pookode Dr Sooryadas S.

Date: (Major Advisor)

CURRICULUM VITAE

PERSONAL INFORMATION

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Present Position:	M.V. Sc. Scholar Department of Veterinary Surgery and Radiology College of Veterinary and Animal Sciences, Pookode, Lakkidi P.O, Wayanad, Kerala – 673 576, Ind

UNIVERSITY EDUCATION

Course	**Institute**	**Board/ University**	**Year**	**Per cent Marks**
BVSc & AH	Veterinary College, Hassan	Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar	2011-2016	8.30

Memberships: Karnataka Veterinary Council

Awards/ Honours Received: 1

Papers/ Posters Presented in National/ International Conferences: 3