Investigation of Biocompatibility, Osteoinductive and Osteoconductive Properties of Clay Cement for Bone Defect Substitution

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Abstract: In the last years, several methods have been used to enhance bone repair and new bone formation. Numerous researches have been focused on possibilities of healing bone defect by using biomaterial implants. Clay cement may represent an important bone substitute and could be used for the treatment of multiple fractures and after bone tumor resection. The aim of this study is to investigate biocompatibility, osteoinductive and osteoconductive properties of clay cement in a rabbit model. Two holes have been drilled in the mid-diaphysis of the femur bone of two animal groups. In the group 1 the defect was left untreated. In the group 2, the defect was filled by clay cement. Radiographs of the defect sites were made immediately after surgery and subsequently on 7, 15 and 21 postoperative days and compared to assess clay cement efficiency. It was concluded that clay cement has osteoinductive and osteoconductive properties and could be used for the treatment of bone defects.

Key words: Clay Cement • Bone Defects • Femur • Bone Substitution • Rabbits

INTRODUCTION

Comminuted fractures of long bones involving varying amount of bone loss are frequently encountered in veterinary practice. Management of such fractures requires not only proper fixation but also maintaining the structural integrity at fracture site by preserving the loose bone pieces. In the cases of bone loss the gold standard method of bone replacement for the treatment of bone gap defects or non-union is the autologous bone graft, where a piece of bone is taken from another body site and transplanted into the defect [1].

Though the success rate of this procedure is quite high, the number of cases in which it can be used are small, due to the limited amount of available tissue and increased risk of donor site morbidity [2, 3]. The second most common treatment is allografting, using tissue from another animal of the same species after processing to reduce antigenicity. This treatment, however leads to a lower rate of graft incorporation with the host tissue [1] and involves the risk of immune rejection and pathogen transmission in the recipient [4].

Therefore, bone tissue engineering has been attracting much attention, nowadays; bone tissue engineering is one of the most important roles in medical science research [5]. Many types of bone filling materials such as different types of calcium phosphate cement have been developed and have played critical roles in bone repair [6] due to their excellent osteoconduction and resorbability [7, 8], but no studies have reported the use of clay cement for filling bone defect.

The aim of this study is to assess radiologically the healing of small femoral defects in rabbits after using clay cement.
MATERIALS AND METHODS

Animals: The study has been carried out by using six adult male local rabbits. Prior to any procedure, animals were housed for 7 days in a temperature-controlled room with 12/12 hr light/dark cycle, fed with standard laboratory rodent chow and drinking water was given ad libitum. Rabbits were divided into two groups of three each: Group 1 (Control) and group 2 (Clay Cement).

Clay Cement Preparation: After harvesting of clay, the cement was prepared by grinding and sterilization at a temperature of 150℃. Cement was then stored in sealed bags until the day of operation.

Surgical Procedure: General anesthesia was induced with an intramuscular injection of 40 mg/kg of Ketamine and 5 mg/kg of Xylazine, then left femur was routinely prepared for aseptic surgical procedure. Animals were restrained in left lateral recumbency and three cm longitudinal skin incision was made on medial aspect of the thigh approximately equally distant from the hip and the knee joint. The space between the biceps femoris and the lateral vastus muscles was dissected with the help of a fine artery forceps, providing a wide review of the femoral diaphysis, of which periosteum was fully dissected (Fig. 1). A segmental bone defect was created in the middle of the femur shaft by drilling two holes, using a pin of 1.8 mm of diameter and saline-cooled in a stepwise fashion (Fig. 2). The defect was then washed carefully with a physiological saline solution. In group 1, defect was left empty. In group 2, clay cement was used to fill the bone defect (Fig. 3). The muscle attachment was then repaired with simple separated 2-0 polyglactin suture and the skin was reapproximated with 2-0 polyamide suture (Fig. 4). Finally, the skin wound was covered by an Aluminum layer.
Observations:

Clinical Signs: Postoperative clinical examinations were performed to evaluate animal response to treatment and the status of cutaneous wound healing were observed in terms of swelling, infection or wound dehiscence.

Radiography: Two orthogonal, medio-lateral and antero-posterior, radiographs of the defect sites were made immediately after surgery and subsequently on 7th, 15th and 21st postoperative days. The radiographs were observed for presence of extent and size of callus, bridging of the gap and reduction of the defect size.

RESULTS

Clinical Signs: The surgical wounds healed by first intention in all the groups. All rabbits have retained their appetite and have not shown any apparent changes after surgery.

Radiographic Observations: On day 0, in medio-lateral views of radiographs, two holes were clearly visible almost at the center of the femur diaphysis in all animals (Fig. 5). On day 7, no evident differences have been noticed between the two groups. A mild periosteal reaction was observed in both group 1 and 2 on day 15, however this reaction was more important in group 2 with a clear gap bridging (Fig. 6), which resulted in a reduction of the defect size. This periosteal reaction was more important on day 21 and size of the bone defect reduced further in group 2 (Fig. 7), but in group 1 the defect size remained almost unchanged (Fig. 8).

DISCUSSION

In cases of fractures associated with bone loss due to high energy trauma or excision of some pathological lesion, the regeneration of the bone is a major difficulty in veterinary practice because the progression of fracture healing has ceased. Progression of healing requires filling of the gap with bone grafts which has been extensively evaluated by several workers with promising results [9-11]. However, various setbacks associated with autogenic as well as allogenic or xenogenic grafts have dictated the researchers to find other alternatives to the bone grafting [12-15]. Despite of lots of advances in the application of various synthetic and natural bone substitutes, problems still exist in the healing of bony defects. An ideal bone substitute should have osteoconductive and osteoinductive properties [16]. The current study aimed to evaluate the positive effect of clay cement on bone response after creating small bone defects in the femur of rabbits. Absence of clinical complication may suggest clay cement biocompatibility; however, advanced biochemical and immunological studies should be performed to confirm such ascertainment.

The formation of bone relies three some crucial processes: osteogenesis (the ability of grafted cells to form bone), osteoinductivity (the ability to modulate the differentiation of stem cells and progenitor cells along an osteoblastic pathway) and osteoconductivity (the ability to provide the scaffold on which new bone can be formed) [17].
In our study, periosteal reaction and gap bridging observed in clay cement treated group demonstrates its osteoconductive and osteoinductive properties and reduction of bone defect size may suggest its osteogenesis property.

The bone cement could be shaped into any complicated defect and filled into any intricate cavity, it could adapt to the bone defect and should provide a good fixation and appropriate contact to stimulate bone ingrowth [6, 18, 19]. The ease of preparation and use of clay cement in this study, make of this natural substitute, suitable for defects of any shape.

In conclusion, the current study successfully constructed femoral cortical bone defect using clay cement. This natural bone substitute can be successfully used for bone healing due to good biocompatibility and its osteoinductive and conductive properties.

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**REFERENCES**

