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REVIEW ARTICLE

Bone defect animal models for testing efficacy of bone substitute biomaterials

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Summary Large bone defects are serious complications that are most commonly caused by extensive trauma, tumour, infection, or congenital musculoskeletal disorders. If nonunion occurs, implantation for repairing bone defects with biomaterials developed as a defect filler, which can promote bone regeneration, is essential. In order to evaluate biomaterials to be developed as bone substitutes for bone defect repair, it is essential to establish clinically relevant *in vitro* and *in vivo* testing models for investigating their biocompatibility, mechanical properties, degradation, and interactional with culture medium or host tissues. The results of the *in vitro* experiment contribute significantly to the evaluation of direct cell response to the substitute biomaterial, and the *in vivo* tests constitute a step midway between *in vitro* tests and human clinical trials. Therefore, it is essential to develop or adopt a suitable *in vivo* bone defect animal model for testing bone substitutes for defect repair. This review aimed at introducing and discussing the most available and commonly used bone defect animal models for testing specific substitute biomaterials. Additionally, we reviewed surgical protocols for establishing relevant preclinical bone defect models with various animal species and the evaluation methodologies of the bone regeneration process after the implantation of bone substitute biomaterials. This review provides an important reference for preclinical studies in translational orthopaedics.

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Introduction

Bone defect healing is a process of reconstruction of the bone tissue, which generally undergoes a multidimensional procedure with an overlapping timeline [1]. The vast majority of bone defects can heal spontaneously under suitable physiological environmental conditions due to the regeneration ability of bone. However, the healing process of bone defect is time consuming, and new bone generation takes place slowly because of decreased blood supply to the fracture site and insufficiency of calcium and phosphorus to strengthen and harden new bone. In addition, large defects, also known as critical bone defects, may not heal spontaneously and lead to nonunion prognosis due to the size of defects or unstable biomechanical properties, unfavourable wound environment, suboptimal surgical technique, metabolic factors, hormones, nutrition, and applied stress [2,3]. Bone grafts or substitute biomaterials are commonly used therapeutic strategies for clinical bone surgery to fill the bone defects for reconstructing large bone segments. Although autografts are the current gold standard treatment for bone defect regeneration [4,5], it still has disadvantages such as limitation in donor supply [6], donor site pain, or haemorrhage [7]. Other disadvantages of allograft are the risk of immune-mediated rejection, the transmission of infectious diseases and the negative effect on the mechanical and biological properties of graft [8—11]. In order to overcome the limitations associated with the current standard treatment of bone grafts, there has been an increasing interest in studying substitutes biomaterials, which are made of naturally derived and/or synthetic materials, during the past decades throughout the world [12—16]. The ideal bone graft substitutes should be biocompatible, bioresorbable, osteoconductive, osteoinductive, structurally similar to nature bone, and easy or ready to use.

Prior to testing in human beings, an ideal bone substitute should be tested both *in vivo* and *in vitro*, so as to make sure that it works effectively and safely. Therefore, to establish a suitable animal model is an indispensable step when evaluating the mechanical property and biocompatibility of bone substitute biomaterials. In this review, we discuss the speciality of different species for estimating bone defect substitute biomaterials in different bone defect sites, such as crania [17—19], femora [20—22], and ulna [23—25]. We evaluated the advantages and disadvantages of each species for estimating specific defects, analysed and compared the similarities between animal models and human clinical situations, and emphasised the factors we need to consider when choosing animals.

General selection criteria

A number of animal test models, such as rat/mouse [26—30], rabbit [31—34], dog [35—38], sheep [39—41], goat [42—44], and pig [45—48], have been developed to simulate human *in vivo* environment and physical conditions to test the availability and comparability of bone substitute biomaterials. In order to mimic various orthopaedic situations, many defect sites have been explored, such as calvaria [17—19], femora [20—22], and ulna [23—25]. A prerequisite for such a model is that no spontaneous complete osseous regeneration of the created defects occurs during the lifetime of the animals [49]. The critical size defect is defined as the smallest osseous wound that does not heal spontaneously over a long period of time. For practical purposes, if there is no mineralised area of $\geq$30% after 52 weeks, there would never be complete bony regeneration. Although the minimum size that renders a defect "critical" is not well understood, it has been defined as a segmental bone deficiency of a length exceeding 2—2.5 times the diameter of the affected bone [11,50].

Various factors have to be considered for selecting a specific animal species as a testing model. First and foremost, the chosen animal model should clearly demonstrate both significant physiological and pathophysiological analogies in comparison to humans. Second, it must be manageable to operate and observe a multiplicity of study objects postsurgery over a relatively short period of time [51]. Other selection criteria include costs for acquisition and care, animal availability, acceptability to society, tolerance to captivity, and ease of housing [52]. According to the international standard, we should also consider the size of the implant test specimens, number of implants per animal, intended duration of the test, and potential species' differences with regard to biological responses [53].

The following are the most frequently used animal models for creating bone defects to test conventional and innovative biological biomaterials to be used as bone substitutes.

Rabbits

Advantage and disadvantage of rabbit models

Rabbit is one of the most commonly used animal models, and it ranks first among all the animals used for musculoskeletal research [54]. However, regarding the assessment of multiple substitute biomaterials, the small size of rabbits is the major drawback for studying orthopaedic implants. However, it was reported that there were similarities in bone mineral density and the fracture toughness of mid-diaphyseal bone between rabbits and human [55]. Besides, in comparison with other species, such as primates or some rodents, rabbit has faster skeletal change and bone turnover [56]. Rabbits are easily available, and easy to house and handle. These characteristics make rabbits the first choice when researchers develop animal model for the *in vivo* test of a new bone substitute biomaterials.

Application of bone defect model for testing bone substitute biomaterials in rabbits

In recent years, several rabbit models have been used to test new bone substitute biomaterials. The most common implantation sites include bilateral tibiae and distal femur (Table 1). Walsh et al [57] investigated three commercially available and clinically used β-tricalcium phosphate (TCP) bone graft substitutes with the same chemistry (Vitoss, Osferion, Chronos), but with various macro- and microscopic characteristics, using a bilateral tibial metaphyseal defect model on New Zealand white rabbits. Bilateral defects (5 mm wide and 15 mm long) spanning the metaphyseal and