The choice of control groups in preclinical bone defect models in rats - A systematic literature review of current literature

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INTRODUCTION: Large bone defects and bone loss are considered one of the biggest clinical challenges for orthopedic surgeons, and multiple clinical indications exist for bone defect repair using bone substitutes.[1,2] Many *in vivo* animal models are used in research focusing on the healing properties of new biomaterials developed as bone substitutes.[3] One of the first-choice models for regeneration of the bone tissue are mice and rats,[3] commonly conducted as femoral defect models.[4,5] The potential efficacies of new bone substitutes can be compared with the baseline effectiveness of the bone regeneration potential with empty defect (negative control) and bone graft (positive control; allograft or autograft). While there is a plethora of new research, many translational challenges exist, and very few artificial bone graft reaches clinical use.[6] We hypothesized that the heterogeneity in preclinical models is detrimental to clinical translation and that the choice of controls (negative and positive) can influence the study outcomes. The objectives of this systematic literature review were to summarize the different control groups used to compare new biomaterials developed as bone substitutes (test items) in preclinical bone femoral defect models in rats and to analyze potential pitfalls related to control groups to improve future preclinical research translation ability.

METHODS: This study utilized the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology to review the scientific literature from 2017 to 2022. A search in PubMed and Embase databases with syntax specific for *in vivo* preclinical studies in rats and bone defect models was conducted to collect all original publications on the subject. Only studies associated with *in vivo* preclinical models in rats and bone defect models investigating a new biomaterial were selected. Data from all included studies were collected into a standardized data extraction sheet, which included: (1) primary author, (2) year published, (3) number of rats included, (4) demography of the rat population, (5) surgical method used, (6) positive and negative control used, (7) test item investigated, (8) origin of the bone graft when applicable, (9) outcomes evaluated, (10) whether or not the test item performed better than the controls used in the study, (11) critical findings regarding inflammation or foreign body reaction linked to bone grafting.

RESULTS SECTION: The literature search yielded 1680 results. After eliminating the duplicates and screening titles and abstracts, 346 potentially relevant articles were retained for full-text review. A total of 157 studies were judged eligible and included in the analysis. The surgical method, control groups, and outcomes were inconsistent across studies. The surgical method most commonly involved the creation of bicortical bone defects (n=63/157, 40%) in the mid-diaphysis femoral region (n=83; 53%) without bone fixation (n=80; 51%). Most studies (n=116; 74%) did not include a positive control group in their experiment. When bone grafts were used as positive controls (n=25; 16%), most of the grafts were allograft (n=12/25; 48%) or autograft (n=8/25; 32%). Less than half of the studies (n=75; 48%) included a negative control group. The control groups used to evaluate the test item impacted the healing comparison, with 84% of studies showing better healing of their test items compared to empty defects (n=63/75; 84%) versus 30% in studies using positive controls (n=12/40; 30%; Figure 1). Overall, 26% (n=41/157) of the studies used bone graft as control and/or test items (Figure 2), which were allograft (n=26/41; 63%), autograft (n=8/41; 20%) or xenograft (n=7/41; 17%). From these studies, 22% (n=9/41) reported an inflammatory and immune reaction linked to the bone graft, potentially impacting their results.

DISCUSSION: In human clinics, autologous bone grafting (harvested from the same individual receiving the graft) is the gold standard for bone substitution in bone defect repair.[7,8] This should be the benchmark for novel bone substitutes and ideally compared with the baseline effectiveness of the bone regeneration potential with empty defect (negative control). The wide variety of control groups described in the included studies and their impact on the outcomes when used to evaluate the test items suggest that they can influence the study outcomes. This finding is important as choosing the wrong or, even worse, no control groups might decrease the clinical translation of novel biomaterials. Furthermore, considering inflammatory and immune reaction from bone graft other than autologous is essential when analyzing the potential bone healing of new biomaterial before clinical implementation.

SIGNIFICANCE/CLINICAL RELEVANCE: The use and standardization of positive controls such as the origin of bone grafts (allogenic, autologous, xenograph) are essential to prevent misinterpretation of preclinical study results and increase the ability to compare the results between studies. Researchers are urged to consider potential pitfalls in their studies to improve future preclinical research translation ability.

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IMAGES AND TABLES:

Figure 1:

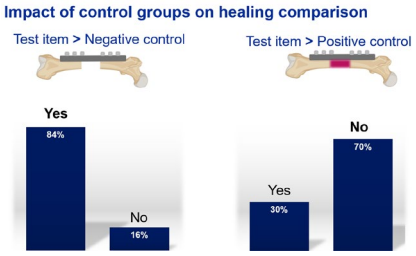

Figure 2: