Biomaterials 30 (2009) 2149–2163

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Leading Opinion

The challenge of establishing preclinical models for segmental bone defect research☆

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ARTICLE INFO

Article history:
Received 13 November 2008
Accepted 19 December 2008
Available online 10 February 2009

Keywords:
Bone defects
Bone engineering
Animal models
Preclinical studies
Orthopaedic research

ABSTRACT

A considerable number of international research groups as well as commercial entities work on the development of new bone grafting materials, carriers, growth factors and specifically tissue-engineered constructs for bone regeneration. They are strongly interested in evaluating their concepts in highly reproducible large segmental defects in preclinical and large animal models. To allow comparison between different studies and their outcomes, it is essential that animal models, fixation devices, surgical procedures and methods of taking measurements are well standardized to produce reliable data pools and act as a base for further directions to orthopaedic and tissue engineering developments, specifically translation into the clinic. In this leading opinion paper, we aim to review and critically discuss the different large animal bone defect models reported in the literature. We conclude that most publications provide only rudimentary information on how to establish relevant preclinical segmental bone defects in large animals. Hence, we express our opinion on methodologies to establish preclinical critically sized, segmental bone defect models used in past research with reference to surgical techniques, fixation methods and postoperative management focusing on tibial fracture and segmental defect models.

1. Clinical background

In general, bone possesses a good healing capacity and the vast majority of bone defects, when stimulated by well-balanced biological and micro-environmental conditions, heal spontaneously. Refinements in surgical techniques, implant design and peri-operative management have significantly improved the treatment of complex fractures and other skeletal defects caused by high energy trauma, disease, developmental deformity, revision surgery, and tumour resection [1–6]. However, an unfavourable wound environment, sub-optimal surgical technique or biomechanical instability can lead to formation of large defects with limited intrinsic regeneration potential [7]. Such defects pose a major surgical, socio-economical and research challenge and can highly influence the patient's quality of life due to limb length discrepancy and prolonged, postoperative treatment courses [8,9].

Even though cancellous bone fractures of the proximal humerus, distal radius or the tibia plateau often lead to impaction

☆ *Editor's Note*: This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

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doi:10.1016/j.biomaterials.2008.12.050

of bone and consequently a defect after reduction [4], the tibia shaft represents the most common anatomic site for segmental bone defects. This is because it is devoid of muscle coverage on its anteromedial surface [8]. The poor soft tissue coverage both increases the risk of bone loss and complicates treatment [8]. Historically, limb amputation was the principal treatment option when facing segmental, non-healing defect sites [10].

Over the years, bone grafts have advanced as the "gold standard" treatment to augment or accelerate bone regeneration [1,2,11–17]. However, significant drawbacks are associated with this approach. Additional anaesthetic time and personnel are needed for graft harvesting [13,15,18]. In many cases, insufficient grafts are obtained and the access to donor sites is limited [13,14,19,20]. Donor site pain or haemorrhage can occur and the donor bone is predispositioned for failure [4,13,14,21]. Moreover, the risk of infection is significantly increased. Graft failures usually result from incomplete transplant integration, particularly in large defect sites [15]. In addition, graft devitalisation and subsequent resorption processes can lead to decreased mechanical stability [22]. Vascularised autografts are technically demanding and allografts and xenografts carry the risk of immune-mediated rejection, graft sequestration and transmission of infectious disease [9,23–29]. The dense nature of cortical bone allografts impedes revascularization and cellular invasion from the host following implantation [19]. This limited ability to revascularize and remodel is believed to be responsible for a failure rate of 25% and a 30–60% complication rate associated with allografts [19,30]. In addition, the maintenance of bone banks is rather costly. A technique introduced to avoid graft integration-related difficulties is commonly referred to as the "Ilizarov technique" which involves osteotomy and distraction to stimulate bone formation. It is used as a treatment modality for large bone defects, infected non-unions, and limb length discrepancy [31]. However, the Ilizarov technique is a long-lasting procedure, highly inconvenient for the patient [32,33] with recurrent pin track infections as a frequent complication [25,34].

In order to avoid the limitations associated with the current standard treatment modalities for segmental bone deficiencies, there has been a continuous interest in the use of naturally derived and synthetic bone graft substitutes during the past decades.

More recently, the concept of tissue engineering has emerged as an important approach to bone regeneration research. Tissue engineering unites aspects of cellular biology, biomechanical engineering, biomaterial sciences and trauma and orthopaedic surgery. Its general principle involves the association of cells with a natural or synthetic supporting scaffold to produce a three-dimensional, implantable construct. To biomechanically simulate human *in vivo* conditions as closely as possible, and to assess the effects of implanted bone grafts and tissue-engineered constructs on segmental long bone defect regeneration, a number of large animal models have been developed. However, reviewing the current literature most of the preclinical models reported in the literature are not well described, defined and standardized. This year, the Journal of Bone and Joint Surgery published a number of review papers on preclinical models in fracture healing and on non-unions [49]. However, these articles provide only rudimentary information on how to establish relevant preclinical segmental bone defects in a large animal model. Hence, the aim of this leading opinion article is to provide both detailed information on the advantages and disadvantages of the different animal models and to comprehensively share the expertise and knowledge of three research groups that successfully established a preclinical animal model for critically sized segmental bone defects.

2. Definition of a critical size bone defect

An experimental osseous injury inflicted to study bone repair mechanisms should be of dimensions to preclude spontaneous healing [35]. Therefore, the non-regenerative threshold of bone was determined in research animal models inducing so-called critical-sized defects. Critical-sized defects are defined as "the smallest size intraosseous wound in a particular bone and species of animal that will not heal spontaneously during the lifetime of the animal" [30,36,37] or as a defect which shows less than 10 percent bony regeneration during the lifetime of the animal [37].

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 [25,34]. Results of various animal studies suggest that critical-sized defects in sheep, however, could be approximately three times the diameter of the corresponding diaphysis [34]. Nevertheless, a critical defect in long bone cannot simply be defined by its size but may also be dependent on the species phylogenetic scale, anatomic defect location, associated soft tissue and biomechanical conditions in the affected limb as well as age, metabolic and systemic conditions, and related morbidities affecting defect healing [25,36] (Table 1).

3. Large animal models in bone defect research

Animal models in bone repair research include representations of normal fracture healing, segmental bone defects, and fracture non-unions in which regular healing processes are compromised without presence of a critical-sized defect site [38]. In critical-sized segmental defect models bridging of the respective defect does not occur despite a sufficient biological microenvironment due to the removal of critical amounts of bone substance. In contrast, in a true non-union deficient signalling mechanisms, biomechanical stimuli or cellular responses may prevent defect healing rather than the defect size.

When selecting a specific animal species as a model system, a number of factors need to be considered. In comparison to humans, the chosen animal model should clearly demonstrate both significant physiological and pathophysiological analogies (Table 2) in respect to the scientific question under investigation prior to animal selection. Moreover, it must be manageable to operate and observe a multiplicity of study objects post-surgery over a relatively short period of time [39–41]. Further selection criteria include costs for acquisition and care, animal availability, acceptability to society, tolerance to captivity and ease of housing [42].

Several publications over the last decades have described dogs as a suitable model for research related to human orthopaedic

Table 1
Factors influencing the quality and quantity of bone healing in long bone critical-sized defects (CSD).

Factors determining a CSD [25,30,36]
○ Age
○ Species phylogeny
○ Defect size
○ Anatomic location
○ Bone structure and vascularisation
○ Presence of periosteum
○ Adjacent soft tissue
○ Mechanical loads and stresses on the limb
○ Metabolic and systemic conditions
○ Fixation method/stiffness
○ Nutrition