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Preclinical testing of drug delivery systems to bone☆

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ABSTRACT

Bone defects do not heal in 5–10% of the fractures. In order to enhance bone regeneration, drug delivery systems are needed. They comprise a scaffold with or without inducing factors and/or cells. To test these drug delivery systems before application in patients, they finally need to be tested in animal models. The choice of animal model depends on the main research question; is a functional or mechanistic evaluation needed? Furthermore, which type of bone defects are investigated: load-bearing (i.e. orthopedic) or non-load-bearing (i.e. craniomaxillofacial)? This determines the type of model and in which type of animal. The experiments need to be set-up using the 3R principle and must be reported following the ARRIVE guidelines.

Contents

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1. Introduction

Large bone defects, including those resulting from trauma and tumor removal surgery, remain a major challenge for clinicians. Current treatment options include autologous and allogeneic bone grafts, with both options demonstrating considerable risk to the patient. Approximately 20–30% of patients who receive autologous bone grafts experience morbidity at the graft-harvesting site, which can lead to significant pain and increased cost due to extended treatment times. Over 30% of patients, who receive allogeneic grafts, exhibit complications, including fracture, non-union and infection [1–3]. In addition, other disadvantages include potential immunogenic responses to host tissue and disease transmission from the donor tissue [4]. Furthermore, it is not possible to harvest sufficient quantities of autograft material from the iliac crest to fill excessively large bone defects, such as those commonly presented in major trauma and osteosarcoma cases. Defects greater than 6 cm may be treated with bone transport or free vascularized bone transfer [5]. Bone transport is the historic gold standard for larger defects, however, complications are common and the process can be laborious for both the patient and the surgeon as patients may be required to use external fixation systems for up to one and half years [5–7]. Pelissier *et al.* reported success when using vascularized free fibula grafts for defects averaging 6–8 cm, however, use of free-fibula graft requires micro vascular expertise that is not readily available at all hospitals and more importantly operation time of 8 to 10 hours [8]. Thus, both of these options are not optimal alternatives as they are expensive, uncomfortable for the patient, and exhibit high risks of complication. Following tumor resection, the only current alternative to bone grafting is through using metal prostheses, including titanium rods. 145,775 bone transplantations were necessary in Europe in 2003. The European market for synthetic bone substitutes amounted to €74 million in 2003. The total socio-economic impact of non-unions in 2008 amounted to €14.7 billion per year [9]. 135,360 bone grafting procedures were performed in the USA in 1998 with a volume of $96.6 million. The worldwide market for bone replacement and repair is estimated at about €300 billion (Concord Corporate Finance Research 2002). Bone diseases are one of the major health problems in Europe and age- and traffic-related complications are both set to rise further. Stem cell treatment is poised to reduce major complications of bone fractures, which might fail to regenerate to a full extent the injured or lost tissue after 9 months, termed non-unions. A conservative estimate of non-union incidence following fracture is between 2 and 10% [10], while others have suggested the incidence to be in the range 3–48% [11]. The incidence of non-union in the US has been estimated at 100,000/year [12]. For these patients, current treatment is inadequate and they will have to live with outcomes, which often include limb amputation. One current treatment uses expensive recombinant Bone Morphogenetic Protein 7 (BMP7) reaching improvements of 40–47% [13,14]. However, BMP shows a cost-effectiveness of only 6.4% [15]. Moreover, the drug delivery properties of the compound are not optimal. Of note, BMP7 is not on the market anymore, as the company did not want to continue to produce it. The only alternative today is BMP2.

Along with trauma, surgical removal of carcinomas is a major cause of large bone defects. Cancer is a major public health challenge. One out of four deaths in the United States are currently due to this disease. In 2012, 1,638,910 new cancer cases and 577,190 deaths from cancer were estimated in the United States [16]. One of the most frequent complications of cancer is bone metastases. Especially in patients with advanced breast or prostate cancer, they occur (70%) as well as in 15–30% of patients with cancer of the lung, colon, stomach, bladder, uterus, rectum, thyroid or kidney [17]. Therefore, reconstruction of critical bone defects is an increasing clinical challenge due to the inadequacy of current treatments. In addition, the use of growth factors, e.g. BMP, following tumor resection is avoided due to their possible interaction with residual cancer cells and their listing as a contraindication on BMP products [18].

Increased road traffic accidents, a higher incidence of obesity, as well as reduced physical fitness results in a more pronounced influence of musculoskeletal illnesses [19]. Moreover, the ageing of the population worsens the situation. Aging is not only associated with a higher fracture risk but also a diminished capacity for bone healing. Failure to effectively treat large segmental defects caused by trauma or tumor resection has significant consequences in terms of quality of life for patients and reduced economic participation [20]. The consequences of failing to restore full function to an injured limb are dramatically demonstrated by the statistic that only 28% of patients suffering severe open fractures of the tibia are able to resume full function and hence return to previous employment [19].

Thus, it is necessary to develop drug delivery systems for providing bone regenerative drugs to the injured site. As part of these developments, *in vivo* testing is necessary before translation to patients is possible. The choice of the animal model is crucial for the success of the developed drug delivery system. Unfortunately, not many drug delivery systems have found their way to the clinical arena in the area of bone regeneration until now. Furthermore, translation potential from the animal model to the patient is not always evident [21]. Therefore, the choice of the animal model is of utmost importance. Sometimes, data from several animals need to be combined in order to get the best possible estimate for translation success. Nevertheless, there is no guarantee that the data obtained in *in vivo* models can be extrapolated to the patient situation. Size of the bones and differences in vascularity may be reasons for that.

2. Bone regeneration

2.1. Localization

When discussing bone regeneration, bone topography needs to be taken into consideration. Different types of bone exist such as flat, short, long, and irregular bones. They develop in two different ways, namely intramembranous or enchondral ossification. As fracture healing is a recapitulation of embryonic development, alternative drugs may be needed for bones of different origin. Moreover, these bones are localized in diverse body areas. They mainly differ in exposure to loading. Flat bones are found in areas without much load bearing, whereas long bones are subjected to mechanical load bearing. This load bearing modulates the remodeling of the bone tissue and it is known as Wolff's law of bone transformation [22–25]. Osteocytes and osteoblasts constitute the cells that respond to mechanical loading in bone tissue. This loading consists of compressive deformation and fluid shear stress