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Large Bone Defect Repair Models Using Cell-Based Constructs: A Translational Lack of Evidence

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Abstract

Despite a constant improvement in the restoration of bony disorders, the treatment of skeletal defects in long bones remains a challenge for orthopaedic surgeons. The available surgical options enable them to restore the integrity of the skeleton, but with considerable morbidity due to a prolonged treatment with multiple surgical interventions in most cases. The holy grail of bone reconstruction by simply filling the defect with suitable biological potent stemcell-scaffold combinations, usually classified as Advanced Therapy Medicinal Products (ATMPs), does not seem to be within a short term reach. Clinical attempts are scarce and often reported as case reports. A uniform protocol does not exist and experimental data are usually heterogenous and not comparable. Moreover their experimental design is not always ideal resulting in data that do not withstand the translation from bench to bedside. This may explain why most scientific results remain silent publications that never make their way to the clinic. The 'ideal' conditions in which the experiments were performed allow for an outcome that cannot be expected to occur in the clinical situation were the biological conditions are less favorable.

Keywords

Skeletal defects; Stemcell-scaffold; Biological condition; Bone reconstruction.

Introduction

One of the first hurdles to take in an experimental setting for studying bone defect repair is the use of a suitable model that mimics the real clinical situation, allowing a "copy-paste" to the patient [1-3].

The studies of ATMPs that can replace defects in long bones under load-bearing conditions do not follow a straightforward protocol [4, 5], resulting in a slow or almost absent progression towards its clinical translation in the last decade. Many studies show apparently good results in usually small animal models, but never evolve to a further stage [6]. Although these investigations are meant to extend our knowledge about quality, safety, feasibility and efficacy, the progress to the human clinical setting appears to be delayed. One of the main reasons is that the models used in the experimental settings are not a blueprint for the surgical application, and will as such not be accepted for use in the operating room. Investigators should be aware that their experimental results can only reach patients if they can be copied by surgeons to their daily practice. The set-up of models mimicking the human situation is of paramount importance, but unfortunately too often shortcomings in the experimental set-up are obvious, predicting their failures towards translation [7].

Bone repair strategies are complex, both with regard to the cell based construct itself as to the receptor area. Ideally ATMPs are assembled with cells, scaffolds and growth factors but for each component a lot of unanswered questions remain.

Whether the ideal type of cell should be bone marrow stromal, periosteal or adipose stem cells has not been established yet [8 -9]. The optimal isolation and expansion techniques differ among laboratories and the exact amount of cells per volume necessary has not been exactly defined [10]. Studies about cell survival show different results and it is not known whether early cell death is beneficial due to the release of growth factors, or not [11]. The best bone promoting scaffold still has to be determined, but it is generally accepted that a calcium phosphate carrier offers the best three dimensional microenvironment [12].

The adherence and distribution of cells on the scaffold and the interaction between the latter and the cells is not always known into depth and the influence of the scaffold on cell behavior often poorly documented [13].

Apart from these shortcomings there are also concerns about the experimental set-up to test the cell-scaffold constructs, as according to literature many studies do not represent the clinical situation and as such have limited translational evidence.

Shortcomings in experimental set-ups

Treatment of 'fresh' defects: In experimental settings defects are created in normal limbs, which unfortunately do not represent the surgical need. In clinical conditions non-unions occur due to complex high energy traumas with soft tissue or vascular damage, infections or iatrogenic conditions such as periosteal stripping, turning the affected area in a poor biological environment. The healing potential is far more compromised than in defects made in 'healthy' long bones and good experimental results in such conditions are absolute no guarantee that the same treatment will work in patients [14].

Immaturity of the treated bone: If experiments are set up to mimic healing in adult patients- who are the common population presenting with bone healing problems-, it is an absolute bias to use skeletal immature animals. Their healing potential is much higher, often leading to spontaneous regeneration, and does not represent a reliable model for the human setting. To our opinion an immature model might be used for the study of bone reconstruction in a pediatric population. However, knowing that traumatic non-unions in children are – with the exception of congenital pseudarthrosis – almost inexistent, these experiments are insignificant for an adult population[15].

Creation of non-critical defects: Investigators must be aware that studies are performed in real critical size defects. Definitions are somewhat different but it is generally accepted that these are defects that will not spontaneously heal throughout the life-time of the animal and of which the length equalizes twice the diameter. Unfortunately a number of experimental set-ups do not meet this criteria and as such are not suitable to serve as a good clinical model. [16 -17].

Underestimating natural healing: Performing animal surgeries always requires good controls. It is not unusual that untreated defects show a significant spontaneous healing tendency and if this background noise is not eliminated false positive results are generated, suggesting that healing is predominantly caused by the treatment [18].

Sample size: Numbers do matter because statistic calculations on very small numbers are less reliable than on large series. Because cell therapies are associated with high cost experimental sample sizes are often limited. Therefore therapeutic strategies must result in a probability almost bordering on certainty before translation to the patient, making surgeons confident that the new therapy can be of great benefit [19].

The inappropriate bone: Using small bones in hand or feet or calvarial bones do not simulate the biological and mechanical characteristics of long weightbearing bones and fail to represent a reliable model. Nevertheless experimental studies are sometimes performed in such conditions, which leads to nice publications on the potential of some treatments to restore 'bone defects' but it is obvious that this will never pave the way to the clinic for the treatment of tibial or femoral non-unions [20].

Incorrect definition of healing: The bone formation obtained in surgical animal models should be interpreted with care, based on good imaging, with as less interference of osteosynthesis material as possible. Good quality radiographs, occasionally completed with CT-scan do offer more clinical relevant information than sophisticated histomorphometric analysis or mechanical testing. Patients will be judged by clinical and radiographic parameters, and accordingly the same evaluation should be done in the experimental animal. A defect reconstruction in a sheep treated with an external fixator that shows complete bone formation and walks pain-free after removal of the frame without any recurrence of fracture can be considered as 100% healed. This is what surgeons need because a treatment should result in a completely healed patient. If there is too much doubt or uncertainty about full bone consolidation caretakers will not be inclined to introduce such a therapy with an unpredictable outcome it in their clinical practice [21].

Xeno or allograft scaffold: Using scaffolds such as xeno- or allograft materials that might induce immunological reactions in the host should be avoided because it is unknown to what extent this influences bone formation. Moreover their use in the clinic is not evident increasing the translational difficulties [22-23].

Discussion

The shortcomings in many studies were already clearly demonstrated by Reichert, about 10 years ago, and a representative ovine model using internal fixation was fine-tuned by his group [24-25]. A similar model with external fixation but starting from a fibrotic defect to mimic the real clinical situation was proposed by our research team [26]. Despite the availability of these suitable models there is still no absolute consensus for using this type of set-up as a standard in translational research for bone defect treatment. Available models and studies remain heterogenous, do not allow to draw a clear conclusion for progression to a clinical application and silently disappear in the archives [27]. One of the most striking observations in literature is the ever returning use of small animal models which can help to sort out whether a tissue engineered construct is safe and worth further examination, but can never serve as a prove of efficacy in the patient. Scaling–up is essential as described in the 'flowchart for translational research' as previously published by our research department [28].

Researchers should be aware of the "cellular paradox" i.e. the fact that cell sizes are roughly the same throughout different species, but that absolute volumes to be filled are many folds larger when animal size increases, necessitating large constructs with hundreds of millions of cells. This upscaling is a challenge for manufacturing the construct as it needs advanced laboratory techniques which should be (partially) automated since this is not manageable by hand only [29].

For clinical application this kind of constructs is a prerequisite which should be tested in a 'human-like' model with large geometrical conditions.

It is obvious that in the last decade only a very slow progress is made towards clinical translation which on one hand is due to the inability to perform the ideal representative experiment. On the other hand the clinical need can be achieved using '*in vivo* tissue engineering' by distraction osteogenesis which allows to create large cylinders of new bone in patients suffering from severe bone loss, and this at a low cost compared to the use of ATMPs. One of the major problems to bring ATMPs into practice is the very high cost for development, translational research in animal models and clinical trials necessary for the final regulatory approach. The missing link between the research and the commercialization due to this financial impact has been described nicely by Hollister as the Valley of Death in 2009, but till now no huge progress has been made [30].

Clinically representative experiments were performed by our research group but at a success ratio in only half of the experimental animals. The enormous cost for running new experiments and the difficulties for full automatization of the production of ATMPs put a severe burden on a quick progress, and risk to lead us to Hollister's valley [31]. For all researchers active in this translational field there is a need not only for scientific and surgical input but also for a decent financial support, which unfortunately does not seem to be a priority for many decision makers. In the meantime laboratories continue to develop ATMPs whose way to the clinic does not exceed a snail's pace. Only a full consensus among researchers about the translational steps to take, testing the ideal ATMP which leads to an excellent outcome will be able to persuade the financial care takers to reactivate the process and create the evidence that is still lacking at the moment.

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