Introduction

The vast majority of fractures progress to union and only a small percentage of them (5–10%) are associated with impaired healing requiring further surgical intervention. Atrophic non-unions are usually characterised from a deprived biological substrate and their management remains challenging. It has been demonstrated that a stable fixation of the non-union site and a simultaneous use of autologous bone graft (ABG), facilitates fracture union with good success rates. Lately, with the advances made in every field of medicine, enhancement of the biology of atrophic non-unions has seen the use of alternatives substances to ABG due to its limited availability and the associated donor site morbidity. Such alternatives have been used as implantation of mesenchymal stem cells (MSCs), growth factors (GFs) such as bone morphogenetic proteins (rhBMPs) or autologous growth factors (AGFs) contained in Platelet Rich plasma (PRP) and scaffolds. Although it is well accepted that MSCs, growth factors and scaffolds are all essential for the evolution of bone healing according to the diamond concept, clinicians have adopted the use of only one of the above options for biological enhancement at the non-union site. This strategy, otherwise known as ‘monotherapy’, has not produced consistent findings and it is of note that in over half of the reported cases ABG has been used with disappointing results.

Polytherapy can be considered as a new strategy of biological stimulation where impaired bone healing is anticipated or it is already established. This strategy would involve the utilisation and simultaneous implantation of all three fundamental components of the diamond concept: MSCs, growth factor and scaffold, assuming that a correct fixation and mechanical stability has been adequately provided.

The aim of this article is to determine the evidence supporting the direct role of MSCs, Growth Factors and scaffolds in the biological enhancement of non-unions and to examine any existing evidence supporting the polytherapy approach.

Materials and methods

Using the PubMed search engine a research of the published series on polytherapy in long bone non-unions was performed at the 10th of August 2010. The following keywords were used: “polytherapy” OR “poly therapy” OR “BMPs” OR “MSCs” OR “PRP” OR “Multi-approach” OR “ABG” OR “Growth Factors”. The exclusion criteria were: case reports or referring to children (age < 16 years); editorials; letters; review studies and articles in languages other than English.

Data from the accumulated manuscripts were collected mainly addressing the issues of principles/stages of management of long bone non-unions, clinical and radiographic outcome and complication rates. When possible, descriptive statistical means were used to comprehensively present the reviewed evidence.
Results

From a considerable number of initially retrieved abstracts on polytherapy, 903 in total, based on the definition of polytherapy as simultaneous application of all three fundamental components of the diamond concept and based on the inclusion/exclusion criteria previously described, ten studies were found eligible to be included in this study.5,7,26,31,40,44,47,71,76,88

In a vitro study the VEGF application with MSCs showed to enhance the osteogenesis process. Moreover, the addition of recombinant human bone morphogenetic protein-2 (rhBMP-2) greatly increased osteocalcin (OC) release from MSCs promoting the healing response.44

Another recent study has illustrated the beneficial effect of bone morphogenetic proteins on the chondrogenic and osteogenic differentiation of human bone marrow mesenchymal stem cells.71

In two animal studies 6,7, the new bone formation in femoral defects of rats was clearly superior in the group treated with the combination of human-MSCs (hMSCs) and rhBMP-7 compared to rats treated with rhBMP-7 or hMSCs alone. The high bone apposition was demonstrated by radiographic analysis and histological examination.

In a dog study, the simultaneous application of rhBMP-7 and MSCs increased the local population of cells and the connective tissue progenitors in a canine femur defect model.76

The association of autologous growth factors (AGFs), contained in platelet rich plasma (PRP), and autologous bone graft (ABG) in a tibial critical size defects of the 16 mini-pigs, was compared with application of autologous cancellous bone graft alone.40

However, the use of platelet-rich plasma (PRP) to improve the bone defect healing is discussed controversially. In a dog model the combination of human-MSCs (hMSCs) and rhBMP-7 compared to rats treated with rhBMP-7 or hMSCs alone. The high bone apposition was demonstrated by radiographic analysis and histological examination.

In a dog study, the simultaneous application of rhBMP-7 and MSCs increased the local population of cells and the connective tissue progenitors in a canine femur defect model.76

The histomorphometrical analysis revealed that the area of new bone formation was significantly higher in the ABG-PRP group. Nevertheless the use of platelet-rich plasma (PRP) to improve the bone defect healing is discussed controversially. In a dog model the combination of human-MSCs (hMSCs) and rhBMP-7 compared to rats treated with rhBMP-7 or hMSCs alone. The high bone apposition was demonstrated by radiographic analysis and histological examination.

In a multicenter study48 of 30 cases of femoral non-unions, healing in 26 out of 30 cases operated with rhBMP-7 was observed. In a subgroup of this study, 12 cases were treated with a combination of ABG and rhBMP-7 and union was successful in 10/12 patients (83.3%). One of these two failures had undergone four previous procedures without success.

Zimmerman et al.88 reported on 26 long bone non-unions of the tibia shaft treated with rhBMP-7. Union was observed in 24/2626 (92%) cases, but after profound analysis of subgroups of this series, it was noted that 8 cases were operated with the simultaneous application of rhBMP-7+ABG and 18 cases with rhBMP-7 alone with or without revision of the osteosynthesis. The union rate in the group treated only with rhBMP-7 was 88.8% (16/18 cases), whereas in the group of combined implantation of ABG and rhBMP-7 it reached 100%.

Finally, in another clinical series, the simultaneous implantation of ABG and rhBMP-7 in 45 long bone non-unions was associated with a success rate of 100% with a median time of clinical and radiographic union of 5 and 6 months respectively.31

Discussion

ABG possess all the three desirable properties that a graft material should retain being osteogenicity, osteoinductivity and osteoconductivity. However, from the practical point of view, prior to the implantation, there is no testing of the autologous graft to investigate whether it does possess all these properties. It is possible that variations that exist from host to host in terms of the presence and the extent of these biological properties could provide a reasonable explanation of the failure rate of cases where the ABG has been used alone as the primary biological stimulus in patients with fracture non-unions. Failure rates have been reported as high as 50%.49 Moreover the harvesting and handling process prior to the implantation could affect the effectiveness of ABG. The reported healing rates where ABG has been used as a biological stimulant for the treatment of long bone non-unions range from 60% to 100%56,27,49,53,88. Additionally, there is universal agreement amongst clinicians that the harvesting process is associated with prolongation of surgery and an incidence of chronic morbidity not to be underestimated.6,39,69,72,87 Moreover, the cost of harvesting can be as high as the cost of implanting a growth factor and it has been argued by some authors that the prolonged surgery time can lead to a proportional increased risk of infection.16,35 These limitations of ABG have stimulated clinicians to apply other biological enhancement options for the treatment of non-unions. But even these alternative strategies have not shown consistently favourable results.2,12,16,19,22,24,27,35,43,47,48,56,82,65,88

Overall, for the treatment of long bone non-unions based on the concept of monotherapy such materials have been used as:

a) Allografts, xenografts or synthetic bone substitutes (HA, TCP, BCP) exhibiting osteoconductive properties. However, such materials require prolonged periods for reabsorption, pose in cases of filling successfully large bone gaps and containment can be difficult. Therefore, they are not ideal when used alone for the treatment of atrophic long bone non-unions.19

b) Demineralized bone matrix (DBM) obtained by demineralization of cortical bone. It has osteoinductive properties due to the presence of “bone morphogenetic proteins” and other factors such as transforming growth factor beta (TGF-β) and insulin-like growth factor (IGF).24 However, DBM has demonstrated a less osteoinductive capacity compared to ABG22 and has shown a high questionable variableness of concentration of BMP-2 and BMP-7 in some products.2

c) MSCs. Studies based on cell therapies have focused on the implantation of concentrated bone marrow aspirate.43,62,27. In addition to their pluripotent properties of plasticity, MSCs are considered osteogenic progenitor cells with demonstrated ability to repair bone defects.6,57,61 However, one aspect that should not be underestimated is the very low concentration of MSCs in the bone marrow tissue.16,41,42,58,59,60,70

d) Autologous growth factors (AGFs) are contained in PRP (platelet rich plasma) obtained by concentration of autologous platelets. It is an advanced product of Blood Management, a biologically active concentrate of mediators extracted from the patient’s plasma that seems to accelerate the normal and physiological healing processes of bone, cartilage and soft tissue.28 It has a similar but much stronger effect as a haematoma, thanks to the high concentration of thrombocytes. Various cytokines (PDGF, TGFβ1-ß2, IGF1-2, VEGF) with a chemotactic, mitogenetic and angiogenetic properties are delivered by degranulation of activated platelets.52 However, recently discouraging results have been reported.1,13,64,85 A randomized study comparing the use of PRP to rBMP-7 for the treatment of 60 long bone non-unions showed inferior results in the group of patients treated with PRP.13 In conclusion, the AGFs contained in PRP, as clarified in previous procedures without success.

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Finally, in another clinical series, the simultaneous implantation of ABG and rhBMP-7 in 45 long bone non-unions was associated with a success rate of 100% with a median time of clinical and radiographic union of 5 and 6 months respectively.31

e) Synthetic growth factors (BMPs). BMPs pose the property of osteoinduction being defined as the phenomenon in which perivascular mesenchymal stem cells are transformed into osteoprogenitor cells capable of forming new bone.14,52,86
Friedlaender et al. reported that the results of treatment of tibial non-unions with rBMP-7 were as good as with the application of ABC.72 Other studies using rBMP-2 have shown superior results of healing compared to ABC.53,37 The clinical evidence about the use of various bone grafts in trauma applications was extensively reviewed in 2007 by De Long et al. After an extensive literature analysis the authors concluded that level 1 evidence to support the clinical use of growth factors exists only for rhBMPs.19 Other studies have also supported the use of BMPs for the treatment of long bone non-unions.12,21,47

In conclusion, rhBMP-7 has a recognized ability to induce the differentiation of osteoprogenitor cells in a pre-chondroblastic and pre-osteoblastic lineage and is able to induce new bone formation.35,52 However, when rhBMP-7 is used alone with or without scaffolds the success rate did not reach levels above 92% (range 75–92%) in 778 consecutive long bone non-unions (Table 1).12,20,27,35,47,56,65

It is clear therefore that the concept of monotherapy, no matter what material is used, has not provided us with the results that one would have envisaged seeing thus far. So, what could be done to improve on the results that we are currently obtaining? The conceptual framework of the diamond concept can be considered as a new strategy for improvement of our results.29,30

The idea of polytherapy, simultaneous implantation of all the three fundamental components (MSCs, growth factors and scaffolds) for fracture healing could be a compelling strategy leading to consistently high success rates.30,82 The existing evidence to support such an approach is currently limited. In some animal studies, the combination of rhBMP-7 with MSCs was found to have a higher osteogenic and osteoinductive power compared to the application of rhBMP-7 or hMSCs alone.6,7,76

For the treatment of human bone defects some investigators suggested that a multi-component approach should be considered.23,66 Should we also consider, besides the implantation of MSCs and a scaffold, the implantation of more than one growth factor?

Given the frequent poor vascularity that often accompanies an atrophic non-union, should we also implant PRP in combination with rhBMPs? Recent studies seem to discouraging the simultaneous implantation of rhBMPs and PRP because the latter is too donor dependent, decreases the osteoinductivity of demineralized bone matrix,63 and is able to suppress the BMPs activity in vitro.38 However, since the vascular endothelial growth factor (VEGF) has been shown to be an important component of the regeneration of the vascular system at the fracture site,43,81 and given the encouraging results of synthetic VEGF in bone defects,50 we wonder if the synthetic VEGF can be useful in combination with rhBMPs. Unfortunately, no studies are available to explore the potential superiority of such a combination.

More recently some human studies have been published reporting on the results of treatment of long bone non-unions using the combination of ABG and rhBMP-7 for biological enhancement with higher success rates compared to the monotherapy approach.31,48,88 However, given the small number of cases treated so far, it is desirable that bigger, more extensive and controlled studies must validate the efficacy of polytherapy.

Monotherapy or polytherapy therefore? In vitro and experimental evidences exist to support a polytherapy strategy.5,7,44,71,76 Such a strategy could potentially accelerate the fracture healing, allow early mobilization of patients and thus reduce morbidity, healthcare costs and complications associated with on-going impaired fracture healing. However, using such a polytherapy approach requires some considerations: What is the ideal timing of implantation of all the materials? Should we implant them simultaneously or at different time points?10,32,80,81

Should we first implant a scaffold, then a week later implant cells when the inflammatory environment has decreased and finally another week later complete the polytherapy concept with the injection of a growth factor? Such an approach however, whilst it might be appropriate, would mean more hospital visits and higher healthcare costs. As the scientific evidence is accumulating it will become clear how the concept of polytherapy can be applied to address successfully all of these concerns. Future studies should take into consideration all these parameters so that clinicians will apply the physiological principles and molecular mechanisms of polytherapy to minimize the potential failure risk in difficult fracture and recalcitrant atrophic long bone non-unions.

Competing interests

The authors have no conflicts of interest to declare. No financial support has been received by the authors for the preparation of this manuscript.

References


Table 1
Overview of rhBMP-7 human applications in non-unions.

<table>
<thead>
<tr>
<th>Study</th>
<th>Date</th>
<th>Country</th>
<th>N</th>
<th>Union (%)</th>
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