

The Use of Growth Factors and Mesenchymal Stem Cells in Orthopaedics

In particular, their use in Fractures and Non-Unions: A Systematic Review

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ABSTRACT

Aim

The aim was to look at current evidence for treating non-unions or delayed fracture healing in regard to novel methods applying mesenchymal stem cells (MSCs) and growth factors (GF).

Methods

Pre-clinical and clinical trials focusing on the use of Mesenchymal Stem Cells and Growth Factors for fracture healing were included in this review. Published articles were identified using specific search terms in Medline, Cochrane Library, PubMed, Scopus and Web of Science.

Results

Of the 580 articles found, 82 met my selection criteria and were included, with 39 papers involving trials on the effects of GFs and MSCs on non-unions or bone repair. These included 11 articles on MSCs, 10 on Bone Morphogenetic Proteins, 2 on Vascular-Endothelial GF, 5 on Insulin like-GF, 4 on Transforming-GF- β , 4 on Platelet-Rich Plasma, 1 on Platelet Derived-GF and 2 on Fibroblast-GF, with the other articles included qualitatively. Overall results were positive with the addition of MSCs, Bone Morphogenetic Proteins, VEGF, IGF and TGF- β in aiding fracture healing compared to controls, with mixed results for other factors.

Conclusion

Overall this review shows promising results regarding the use of MSCs and various Growth factors in the treatment of fractures and non-unions, as well as synergistic effects observed when combined together. However more research is indicated as these methods are still in the early stages of development.

KEY WORDS: Bone Morphogenetic Proteins, Fracture, Growth Factor, Mesenchymal Stem Cells, Non-Union, Orthopaedics, Vascular Endothelial Growth Factor.

1. Introduction

Many of the current interventions in the orthopaedic field revolve around repairing or replacing damaged tissues with long-lasting, compatible materials with few complications and which are cost-effective. Numerous studies have been conducted in the new area of tissue regeneration, and at the forefront of this research is the use of stem cells. Stem cells are undifferentiated cells which have the potential to become any specialised cell; they are effective and have extensive potential for biomedical research[1]. Mesenchymal stem cells (MSCs) in particular are effective as they have the ability to renew and maintain their multipotent nature throughout numerous proliferations, with a large number of cells being cultured from only a small sample of bone marrow[2]. MSCs have been recognised for years for their potential use in bone grafts and this has led to the identification of a number of sources. These include bone marrow, peripheral blood, adipose tissue, teeth and umbilical cord [3,4]. These stem cells in particular have the potential to divide into many mesenchymal lineages (such as bone, cartilage, muscle)[5,6] when under the right conditions[7], making them invaluable for various processes and ideal where immediate applications and increased cellularity may crucially quicken the healing processes[8]. MSCs have been studied from the 1960's and have since been used in tissue engineering to aid in the creation of a scaffold; a 3D construct of living tissue seeded with stem cells that will increase tissue repair once implanted[2]. They have also been used in various other applications such as direct MSC injection and gene-modified MSCs, but for bone non-unions and large defects MSC seeded scaffolds have been most successful[9].

Advancements in biomaterials has allowed the development of scaffolds to enhance regeneration in large segmental bone defects and many materials have been researched, including combinations of MSCs, endothelial cells and growth factors. These additions have allowed progress and have overcome the drawbacks found previously in bone grafting procedures [10]. The currently used grafts include autografts and allografts; autografts are considered the 'gold standard'[11] as they involve cells from the same patient at a different site and these grafts include a lattice structure, growth factors and osteoprogenitor cells. However their disadvantages involve donor site morbidity, extended operating times, lack of a vasculature and an increased risk of nerve or vessel injury and infections. Allografts were introduced to overcome the downside involving donor site morbidity as they can be made in specific quantities from other people's cells and are called 'banked bone'[12]. However these grafts have increased immunogenic responses, are more expensive and have similar drawbacks revolving around lack of blood supply and increased fracture complications and non-unions[13].

It is important to understand the process of fracture healing in order to properly decide which steps to target or which factors to investigate specifically. Fracture healing occurs in four main phases[14] but the basic steps of this process include 'haematoma, inflammation, angiogenesis, chondrogenesis to osteogenesis and bone remodelling'[15]. Angiogenesis is essential and occurs in the early stages of fracture healing, when the haematoma occurs. This is where inflammatory cells, fibroblasts, stem cells and growth factors are involved. It is important to remember that along with stability of the site, vascularisation is crucial for a successful outcome in healing fractures[6]; without which the area of injury would fail to regenerate and die[16]. The sites that are most prone to non-unions are those where there is a limited vasculature and therefore an inadequate supply of vital proteins, cells and growth factors. These sites include the head of the femur and the wrist bones [6] but also the tibial shaft which is a common place of injury and delayed healing.

The use of growth factors in orthopaedics has also been the subject of important research in this field in the last few years. Their use in bone repair is widely known, with many pre-clinical trials but with only limited numbers of clinical trials and therefore less available evidence for their current use in helping with orthopaedic treatments. The main growth factors which are recognised as key in the process of bone healing and remodelling after fracture are bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGFs), transforming growth factor beta (TGF- β), platelet derived growth factor (PDGF) and fibroblast growth factors (FGF)[17].

In this review we will analyse the available literature regarding the application of mesenchymal stem cells and various growth factors on bone formation, fracture healing and non-unions. According to Garrison *et al.*, a non-union is defined as a fracture that demonstrates motion at the bony end and which has not healed completely by 6 months[18], but other definitions state it is when there has been no sign of further healing for at least 3

months. However, it is difficult to set an exact time limit to classify these fractures [19]. These non-unions have been shown to have depleted signalling of essential growth factors in bone healing such as TGF-B and FGF[20] and significant reductions in BMP-2 expression was found in the cartilaginous areas of non-healing fractures[21]. Non-unions have been reported to occur in a range from 4-10% of fractures and they can lead to various morbidities; including severe pain, decreased function and ability to return to work and have a negative effect on quality of life[18] as they may require further procedures and longer hospitalisations [13]. Delayed union or non-unions can also cause pseudo arthrosis and inability to weight bear or walk because of pain [22]. Therefore it is imperative that these fractures are dealt with appropriately and as quick and successfully as possible, so it is important to trial various alternative methods such as the use of MSCs and Growth factors as they may prove to be a successful direction for future therapeutic applications.

2. Aims

The aim of this review was to look at current research and evidence for the use of mesenchymal stem cells and various growth factors on the treatment of fractures and non-unions. We wanted to assess if there were any benefits or adverse effects in the potential application of these factors in the orthopaedic field.

3. Methods

For the purpose of this systematic review we followed the revised PRISMA guidelines (2009) by Moher *et al.*[23].

3.1 Search strategy

We searched a range of online databases including OVID/MEDLINE, Cochrane library, Web of science, Scopus and PubMed. We used the following search terms ‘Growth factor*’, Mesenchymal stem cell* OR MSC* OR stem cell*’, ‘Orthopaedic*’ and ‘Fracture*’ also to narrow down certain searches we searched specific growth factors such as ‘BMP* OR bone morphogenetic protein*’, ‘VEGF OR vascular endothelial growth factor*’ or ‘Fracture OR non-union’. We also performed hand-searches for articles with similar titles to my review and searched relevant article reference lists to try to broaden my search.

3.2 Selection criteria

Studies were selected based on the following inclusion criteria:

1. Study type included preclinical trials, clinical trials and relevant reviews (excluding case series or reports)
2. Studies focusing on the use of mesenchymal stem cells or growth factors in the orthopaedic field
3. Studies also focusing specifically on their use in fractures or delayed healing/non-unions
4. No limitations were placed on the type of growth factors used
5. No limitations on publication year
6. No limitations on subjects of studies. Included both human (clinical) and animal or cell based (pre-clinical) trials.
7. Limitations set on articles having full text available

3.3 Selection of studies

To select my studies we performed a search in each of the databases using my keywords, and from here we scanned all of the titles that were found. In total there were 626 papers found in my search from the various databases and 16 more found by hand searching separately. We subtracted 62 duplicates across the databases and made a note of all relevant titles for my review (580). We selected 277 articles from the title screen to read their abstracts and assess if they were still relevant, 150 of these abstracts were. We then attempted to access the full text of all the papers selected by their abstract and read the full articles. We applied my inclusion criteria to each of them, and included all that were relevant and accessible in my review (82), excluding those that did not fit the criteria (68). In total we found 39 papers with quantitative data and 43 papers with qualitative data. See figure 1 below for my PRISMA flow diagram and a breakdown of the studies found.

3.4 Outcomes

My primary aims were to assess the current evidence on the use of growth factors and mesenchymal stem cells in orthopaedics, in relation to treatment and repair of fractures and non-unions. My secondary outcomes were to find out which growth factors had been trialled before in both pre-clinical or clinical trials and the mode of actions for each of the growth factors and also the various cell sources for mesenchymal stem cells.

3.5 Data collection

This was extracted independently by one author, and information extracted included the types of studies done, types of growth factors and stem cells and potential adverse effects as well as benefits. We included articles with both qualitative and quantitative data.

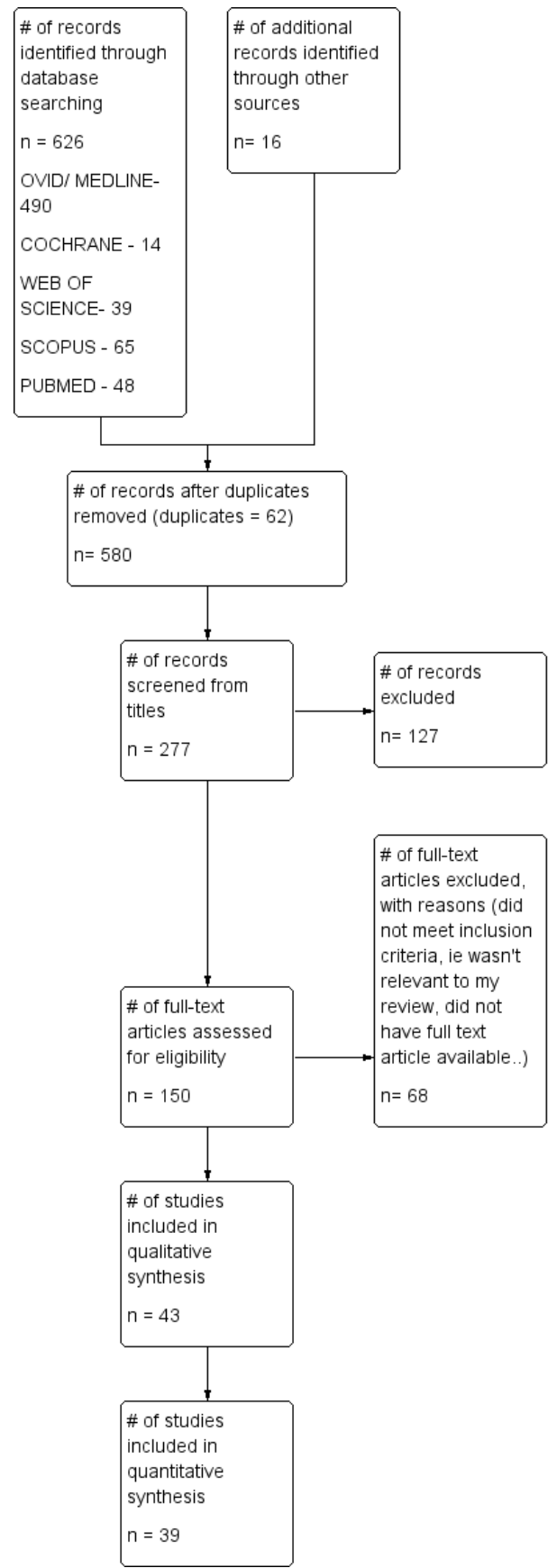


Figure 1: PRISMA flow diagram

4. Results and discussion

4.1 Mesenchymal Stem Cells (MSCs)

It is important to trial allogenic scaffolds with MSCs as an alternative to the gold standard of autologous grafts in bone regeneration. Liu *et al.* studied the efficacy of allogenic mandibular scaffolds and allogenic scaffolds loaded with MSCs in beagle dogs[24]. Here the animals received mandibular defects and were divided into two groups and assessed routinely throughout the 48 weeks. CT examinations showed that by 48 weeks the allogenic MSC loaded scaffolds had been completely replaced by new bone and this surface area was smaller than the original indicating resorption of bone had occurred, whereas in the control group the size of the scaffold stayed the same as the original meaning little new bone was formed. By 12 weeks the bone mineral density was significantly higher than the control group (0.55 to 0.39) and on histological analysis trabecular bone growth was only observed in the experimental group.

Ceramic-based synthetic bone substitutes offer an alternative to allograft and autogenous bone grafts. They are based on hydroxyapatite (HA) and tricalcium phosphates and have already been used in clinical practice [12] because of their useful bone induction properties[9]. Positive results have been seen regarding MSCs and these alternatives, for example Ochi *et al.* reviewed ‘interconnected porous calcium hydroxyapatite’ (IP-CHA) in bone and found along with MSCs it could improve the osteoconductivity and could be used in larger bone defects; they subsequently demonstrated successful bone formation in a study of MSC-IP-CHA in rat tibial condyles [25]. Wang *et al.* also looked at similar B-tricalcium phosphate scaffolds combined with MSCs and the effect of using a pre-vascularised version in segmental bone defects. They tested the experimental combination against a control of just MSCs in the femurs of rabbits bilaterally. They found that at all times analysed in the study the pre-vascularised bone graft had higher volumes of new bone and increased infiltration of capillaries compared to the non-vascularised grafts[26]. This could provide an answer to improve vasculature in MSC based scaffolds.

It is well known that MSCs play a role in the induction of bone formation, but Kallai *et al.* carried out a study to identify any further roles carried out by MSCs in bone repair. They genetically engineered MSCs and investigated the implantation of these cells on the change in microarchitecture of mouse radial bone fractures. They used micro-CT at 10 and 35 weeks to assess the changes compared to limb fracture without MSCs, and results showed significant bone remodelling of limbs implanted with MSCs, accounted for by a large decrease in bone volume and an increase in mineral density[27] indicating a further role for MSCs other than induction.

A few studies looked into the addition of molecules other than growth factors on MSCs and the outcome on fracture healing. It has been postulated as to why the presence of stem cells is so much higher in an injured site

compared with normal tissue. It is thought that MSCs derived from the site of injury recruit other stem cells which sense the injury and migrate towards it. Ho *et al.* hypothesised that certain chemo-attractants played an important role in the migration of these MSCs to an injury; they looked at Stromal cell-derived factor-1 and the effect of MSC's expressing it and bone repair. Rat bone marrow MSCs (rBMC) were harvested and then used in 18 3mm femur fractures of other rats. The study had 3 groups; rBMC infected with SDF-1 cells, rBMCs alone and the control. In the intervention groups the cells were seeded onto collagen sponges and transplanted into the gaps, the control had sponges without cells. The bone mineral content (BMC) was measured at the 1st, 3rd and 6th week and the rBMC-SDF-1 group was found to have a significant increased BMC than the control and the rBMC only groups ($p=0.003$ and 0.0029 respectively). Histology at the site 3 weeks in showed new bone formation in all groups, but the largest increase in the SDF-1 group; significantly more than the rBMC group of MSCs alone surprisingly and not significantly more than the control. They also carried out a migration assay to see whether SDF-1 successfully increases cell migration toward the infected cells. The results showed a dose-dependent relationship between SDF-1 and chemo attractive activity as more cells migrated with higher doses[7].

Qi *et al.* also studied the effect of another factor with MSCs, and here they looked at simvastatin combined with MCS sheet transplantation in bone formation. They looked at the response of healing demonstrated by the release of BMP-2, alkaline phosphatase, VEGF and callus formation; the group including MSCs with simvastatin showed significantly higher expressions of the factors mentioned above and at 8 weeks complete bone fusion was obtained. In contrast groups containing two out of the three parts to the experimental group showed partial fracture bridging whereas the control still showed non-union. These signify the potentially enhanced effects of MSC's with other factors for non-unions [28].

MSCs have been successful in demonstrating osteogenesis actions, but regarding angiogenesis it is thought that growth factors are needed in addition to stem cells, particularly VEGF, to produce successful vasculature [16]. Kumar *et al.* demonstrate this as they found successful neoangiogenesis around the bone defect when treating non-unions with MSCs expressing BMP-2 and VEGF compared with MSCs alone [29]; these factors were found to act synergistically. To overcome this problem in this type of stem cell Correia *et al.* looked at the potential use of adipose derived stem cells and their individual potential for angiogenesis when stimulated appropriately by factors already present in fracture sites. They were attached to scaffolds and subjected to various applications of growth factors and different conditions to induce osteogenesis and angiogenesis at different times and also simultaneously. They found their data strongly supported the conclusion that adipose stem cells could be used as a single source for forming vessels in bone tissue as by week 5 they had evidence of vascular network formation by the presence of endothelial cell surface markers and von Willebrand factor [30], and thus adipose tissue MSCs could exceed the use of bone marrow MSCs in this respect. Li *et al.* further discussed the positive outcomes to large bone defects in large animals, where adipose derived MSCs modified by BMP-2 had a significant effect[31].

Furthermore other studies have looked into varying the sources of MSCs or the application of them to enhance their effect on bone repair. Although in tissue engineering human bone marrow stem cells are the most commonly used site [32,33], alternative sources other than bone marrow MSCs are under investigation[34]; for example human umbilical cord MSCs (h-UC-MSCs), which were looked at with blood plasma on bone regeneration in rats by Qu *et al.*. They showed that these cells could successfully heal non-union fractures and fracture site density was further enhanced by the addition of blood plasma[35]. Alternatively a study recently conducted by Rapp *et al.* looked into the potential success of systemically delivering bone marrow MSC's. After injecting fluorescent labelled MSCs into mice subjected to femur osteotomies they detected the cells in the early and late fracture callus and SDF-1 was strongly expressed at the fracture sites. This factor has been suggested to increase cell migration to an injured site. They also induced mechanical ulnar loading to induce bone formation in the mice, but they failed to detect any labelled MSCs in these sites; concluding the potential application of systemically delivered MSCs to bone injury only. It was not compared to the local application of MSCs though so it is difficult to draw appropriate conclusions and it is still debated whether this technique actually aids fracture repair once MSCs are recruited [36]. It's important to note that all of the studies we assessed for MSCs were pre-clinical, and current research doesn't seem to have reached the clinical phase of trials on humans.

See table 1 below for a summary of trials involving MSCs.

Type	Study	Design	Cell source	Application	Summary
Pre-Clinical	Liu <i>et al.</i> (2014) [24]	<i>In vivo</i>	Canine bone marrow stem cells	Canine mandibular defects	At 48 weeks the allogenic mandibular scaffolds of the experimental group with autologous MSCs had been completely replaced by new bone and decreased in size, but the control group remained the same size throughout. At 12 weeks the bone mineral density in the MSC group was significantly higher than the control (P<0.05).
	Ochi <i>et al.</i> (2014) [25]	<i>Ex vivo</i> and <i>In vivo</i>	Adult peripheral blood and bone marrow	Rabbit ulnar defects	Results suggested a potential clinical use of magnetically labelled MSC for treatments in delayed unions, non-unions and bone defects, as this method of delivery promoted cell accumulation and proliferation at the fracture site. Study was not solely focused on bone applications as they also looked at cartilage, ligaments, muscles and nerves.
	Wang <i>et al.</i> (2010) [26]	<i>In vivo</i>	Bone marrow of rabbits	Rabbit femur osteotomy	The experimental group had prevascularised bone grafts seeded with MSCs and inserted with a vascular bundle into the osteotomy. This had a significantly higher volume of regenerated bone and capillary infiltration compared with the control, which was MSC scaffold alone and non-vascularised. VEGF was also expressed at a higher level in this group than the control group throughout the study.
	Kallai <i>et al.</i> (2010) [27]	<i>In vivo</i>	MSCs from mice and genetically engineered	Radius of mice	Results show that regenerated bone tissue remodels over time, with decreased total volume but increased mineral density. The axial stiffness of limbs with a non-union repaired with MSCs was 2 to 1.5 times higher compared to the contralateral intact limbs, at 10 and 35 weeks after treatment, with overall superior biomechanical properties.
	Ho <i>et al.</i> (2014) [7]	<i>In vivo</i> and <i>in vitro</i>	Rat bone marrow MSCs	Rat bone defects	<i>In vitro</i> they showed that SDF-1 secreted by the transfected stem cells increased the migration of nontransfected cells. In the rat defect bone model bone marrow MSCs overexpressing SDF-1 had significantly more new bone formation in the gap and less bone mineral loss. SDF-1 was concluded to have an important role in fracture repair.
	Qi <i>et al.</i> (2013) [28]	<i>In vivo</i> and <i>in vitro</i>	Rat bone marrow MSCs	Rat tibia osteotomy	Tibias were harvested at 2 and 8 weeks, and showed increased expression of BMP-2, Alkaline phosphatase, osteocalcin, osteoprotegerin and VEGF in simvastatin-induced MSCs and this further increased with higher concentrations of simvastatin, significantly higher than the group with MSCs alone. Results show that both contributed to the complete healing of the tibia.
	Kumar <i>et al.</i> (2010) [29]	<i>In vivo</i> and <i>in vitro</i>	Mice bone marrow MSCs	Mouse tibia bone defects	Increased bone formation in group with BMP-2 and VEGF expressing MSCs. Increased vascularity and osteoblastogenesis compared to control.
	Correia <i>et al.</i> (2014) [30]	<i>In vitro</i>	Human adipose derived stem cells (hASC)	Applied to scaffolds	hASC were inserted with fibrin hydrogel and a porous sponge to form a scaffold, and subjected to various applications of growth factors. By 5 weeks of culture bone development was evidenced by certain markers such as calcium deposition and bone matrix proteins along with vascular networks evidenced by endothelial cell surface markers. Both support the use of adipose stem cells as a source of vascularised bone tissue.
	Li <i>et al.</i> (2007) [31]	<i>In vivo</i> and <i>in vitro</i>	Adipose cells from canine bone marrow	Canine ulnar defects	Adipose cells were genetically modified by BMP-2 and applied to B-tricalcium phosphate carrier and implanted into bone defects. At 16 weeks analysis showed the modified adipose cells produced significant amounts of newly formed bone and healed most of the bone defects.

	Qu <i>et al.</i> (2012) [35]	<i>In vivo</i>	Human umbilical cord MSCs	Rat tibia non-union	Benefits seen in the group treated with these MSCs and blood plasma, with higher fracture site density and a low immunogenicity which merged with rat bone tissue and completed the healing of non-unions. However results were not significant and were similar to a control.
	Rapp <i>et al.</i> (2015) [36]	<i>In vivo</i>	Mice bone marrow MSCs	Mice femur osteotomy or non-invasive mechanical loading of ulnar	Fluorescently labelled MSCs were injected systemically into the mice. These were detected in early and late fracture callus (day 10 and 21) in the femur osteotomies, with a strongly expressed SDF-1 which mediates cells to the injury site. There was more bone in the callus of these mice compared to control however the bending stiffness was not altered. They failed to detect the labelled MSCs in the ulnar sites where mechanical loading had occurred with no bone defect, concluding that these cells are only recruited in injury-induced and not mechanically induced bone formation.

Summary and characteristics of trials involving Mesenchymal Stem cells: Table 1

4.2 Bone Morphogenetic Proteins (BMPs)

BMP's are the main group of growth factors that act on the skeleton, as their key actions involve migration of osteoprogenitors and osteoinduction and proliferation [17]. They have been extensively researched for these properties and have been studied in the context of new bone formation and as an alternative to the current 'gold standard' of autografts for non-union in fractures and bone defects [37,38]. Only recombinant BMP-2 and BMP-7 have made it past clinical trials and into practice [17,39]. BMPs have also been studied in a number of preclinical studies when they were first introduced as a potential aid to promote bone formation, and the benefits of BMP-2 in terms of increased torsional toughness and total callus new bone formation were demonstrated on tibial fracture healing in goats [40]. BMP1-3 effects in rabbit and rat models were discussed by Grgurevic *et al.* who found that BMP1-3 (which is found in surrounding plasma and an isoform of the BMP-1 gene) was significantly increased in acute bone fracture and found in the surrounding plasma and therefore hypothesised that BMP1-3 played a crucial part and also demonstrated that when an antibody was used to neutralise their effects there was delayed bone union [41]. A recent study on the therapeutic potential of BMP-9 on MSCs has also demonstrated significant cross-talk with other signalling pathways inducing trabecular bone and increasing osteogenic markers so potentially could be considered for future clinical use, however more trials are needed with BMP-9 before assessing its clinical applications [42].

BMP-2 has been seen to be advantageous for bone regeneration of acceleration of fracture healing and a number of studies have assessed the benefit in a clinical setting. Govender *et al.* carried out a large prospective randomised study on 450 patients to evaluate the effectiveness of addition of recombinant human BMP-2 (rhBMP-2) on healing of open tibial shaft fractures [43]. Patients were randomised to receive either the standard of care for this injury (which is intramedullary nail fixation) or the experimental groups which were standard of care with an implant containing either 0.75mg/mL dose of rhBMP-2 or 1.5mg/mL dose contained in an absorbable sponge. In their results they found that at the end of the 12 month follow up the group with the higher dose of rhBMP-2 had a reduction in risk of failure of 44% and this was found to be significant ($p=0.0005$). A reduction of failure was measured by the need for secondary intervention because of fracture non-union; such secondary interventions are associated with higher patient morbidity and reduction in quality of life. The rhBMP-2 group with 1.5mg dose had 26% of the patients needing secondary interventions while the lower dose rhBMP-2 (0.75mg) and control group had higher proportions of patients requiring this (37% and 46% respectively). The higher rhBMP-2 group had fewer interventions and complications such as pain, and were also shown to have evidence of faster healing, which was assessed by independent surgical and radiological opinions. The higher dose group had evidence of healing starting at 10 weeks and increasing so that at 6 months there was a 21% increase in healing rate compared to the control group. However infection rates were no different between the groups and surprisingly whilst fracture healing was observed in 50% of the patients at the shortest amount of time in the 1.5mg group at 145 days, the lower dose of rhBMP-7 at 0.75mg

had a prolonged average time compared to the control (187 compared to 184 days respectively) indicating no added benefit at the lower dose. Overall the differences in the results found were significant in terms of the higher dose of rhBMP-2 and the effects were concentration-dependant [43]. These results were also reiterated by Swiontkowski *et al.* in 2006 in a subgroup analysis[44], and by Wei *et al.* in a meta-analysis of rhBMP-2 in open tibial fractures[45]. A health economic analysis of the use of BMP-2 in severe open tibial fractures (grade III) found that in all three countries analysed (UK, Germany and France) savings were made in terms of more secondary interventions due to delayed fracture healing or infection if rhBMP-2 was not used [46]. Overall, it would seem the use of rhBMP-2 is beneficial in many aspects.

BMP-7 is also known as osteogenic protein-1 (OP-1) have been studied by many, including Friedlander *et al.* [47] and Ristiniemi *et al.* who investigated the effects of rhBMP-7 on accelerating fracture healing [48]. OP-1 was first implanted successfully on a patient in tibial non-union over 20 years ago and since then has been trialled extensively. OP-1 has been found to be safe without adverse effects and successful as an alternative to the normal standard of autogenous bone harvested from the iliac crest with improved functional outcomes, such as being able to weight-bear without pain earlier on than control groups [49]. BMP-7 has many clinical uses, mainly studied in terms of non-unions and fracture healing, but it has also been used in other procedures including acetabular reconstruction and enhancement, distraction osteogenesis, free fibular graft and arthrodesis of joints. In a large observational study looking into its applications the overall success rate after application of BMP-7 in persistent atrophic non unions and other procedures was 82% [50].

Ristiniemi *et al.* conducted a trial of 20 patients with distal tibial fractures treated by external fixation and osteoinduction with rhBMP-7, compared with 20 matched control patients. They found significantly more fractures had healed by 16 and 20 weeks in the experimental group than the control. The mean time to union in weeks was 15.7 in the BMP group versus 23.5 in the control group, and this difference was significant with a p value of 0.002. The study also showed a smaller secondary intervention number (2:7) although it was a small sample size which could impact upon statistical power of the trial[48]. Bilic *et al.* also looked at OP-1 (BMP-7) but in the healing of scaphoid non-unions with proximal pole sclerosis and randomly assigned a small sample of patients (17) to 3 different treatment groups: autologous iliac graft, the same with OP-1 and allogenic iliac graft with OP-1. Clinical and radiographic assessments were performed and overall the addition of OP-1 to the first group reduced the radiographic healing time by 5 weeks (4 weeks compared to 9) [51].

In contrast to these results Friedlaender *et al.* conducted a randomised control study included 124 tibial non-unions and treatment of intramedullary rod with rhOP-1 or with bone autograft. They assessed the severity of pain at site, ability to weight-bear and walk, and the need for surgical re-intervention. Results showed that both groups were successful and comparable but whilst 75% of the OP-1 group demonstrated radiological evidence of bone bridging at 9 months, the control group had more success at 84%. However surgical re-treatment occurred in a lower percentage of OP-1 group compared to the control (5% to 10%) [47].

Vukicevic *et al.* recently undertook a review on the use of these two BMPs and in the context of a new carrier device OSTEOGROW for aiding in the clinical use of BMPs in bone healing[52] as it has been found that BMP-2 and BMP-7 when unbound can cause bone formation in surrounding tissues and inflammation in the bovine collagen carriers. But when BMP-6 was attached to this whole blood compatible device it was found to accelerate healing of critical size defects in animals without the adverse effects. It's success has been discussed in other reviews[53].

Regarding the use of MSCs with BMPs together in the treatment of non-unions the 'diamond concept' was introduced in recent years, which incorporates the concurrent use of MSCs with Growth Factors and hormones, scaffold and mechanical stability [54]. The concept has been shown to be successful when applied to treating critical-size bone defects [55] and further studies by Scaglione *et al.* and Giannoudis *et al.* on this method on long bone non-unions both found that the method was valid [54]. It was also found that in subtrochanteric atrophic nonunions which were complicated it allowed optimisation of the environment needed to support healing[56]. Similarly Calori *et al.* analysed the diamond method on 52 patients with forearm non-unions randomised to either 'polytherapy' using all of the components of the concept versus 'monotherapy' with only one. Results showed a higher percentage of non-unions that developed radiographic and clinical healing in the

polytherapy versus monotherapy group (89%: 64%). The average time to clinical union was also prolonged in the monotherapy group (on average 5.29 months compared to 3.65)[57]. However it is unclear how many in the monotherapy group was assigned to either MSCs, rh-BMP-7 or a scaffold.

See table 2 below for a summary of trials involving BMPs.

Type	Study	Design	Application	Summary
Pre-Clinical	Welch <i>et al.</i> (1998) [40]	<i>In vivo</i>	Goat Tibial Fractures	RhBMP-2/ACS group had increased radiographic healing scores, increased torsional strength and stiffness. Total callus new bone volume was significantly increased.
	Grgurevic <i>et al.</i> (2011) [41]	<i>In vivo</i> and <i>In vitro</i>	Rodent Long Bone (systemic) and Rabbit Ulna (local)	BMP1-3 enhanced bone healing in critical sized defects. BMP1-3 increased the expression of collagen and osteocalcin and enhanced mineralisation <i>in vitro</i> in osteoblast cells.
Clinical	Govender <i>et al.</i> (2002) [43]	<i>In vivo</i>	Human open Tibial fractures	rhBMP-2 (1.50mg) group had 44% reduction in healing failures, significantly fewer interventions and faster fracture healing than controls. A significant difference was not found with the lower concentration of rhBMP-2 (0.75mg) compared to controls.
	Swiontkowski <i>et al.</i> (2006) [44]	<i>In vivo</i>	Human open Tibial fractures	Subgroup 1) of severe (type III) open fractures had significant improvements in the rhBMP-2 group, fewer bone-grafting procedures, fewer secondary interventions and lower rates of infection. No difference in subgroup of reamed intramedullary nailing
	Friedlander <i>et al.</i> (2001) [47]	<i>In vivo</i>	Human tibial non-unions	9 months, 81% BMP-7 group and 85% control (autogenous bone) were treated successfully. Radiographically control was higher % healed
	Ristiniemi <i>et al.</i> (2007) [48]	<i>In vivo</i>	Human tibial fracture	RhBMP-7 group had significantly increased fractures healed by 16 and 20 weeks. Time to union was decreased in this group. Delayed healing and secondary intervention occurred in 2 patients of the BMP group and 7 in the control.
	Bilic <i>et al.</i> (2006) [51]	<i>In vivo</i>	Human Scaphoid non-union	BMP-7(OP-1) improved autologous and allogenic bone implants in the non-unions and reduced radiographic healing time from 9 weeks to 4. Increased vascularisation with the addition of BMP-7 was observed compared to the control (autologous graft without addition of BMP-7)
	Scaglione <i>et al.</i> (2014) [54]	<i>In vivo</i>	Human long bone non-unions	Tested the 'diamond concept' of MSCs and BMPs on non-unions and found complete healing in 78.9% (15 cases) with an average healing time of 6.5 months. However there were no controls to compare the outcome.
	Giannoudis	<i>In vivo</i>	Human non-	82% success rate with BMPs in treatment of

	<i>et al.</i> (2013) [56]		unions	fracture non-union. No local or systemic effects were encountered and both clinical and radiographical union was seen.
	Calori <i>et al.</i> (2013) [57]	<i>In vivo</i>	Human non-unions	RhBMP-7 vs PRP: clinical and radiological union in 87% of rhBMP-7 compared to 68% in PRP, and a lower clinical and radiographical healing time for the BMP-7 group (3.5 vs 4 months, and 8 vs 9 months)

Summary and characteristics for trials involving Bone Morphogenetic Proteins: Table 2

4.3 Vascular Endothelial Growth Factor (VEGF)

The main biologic effect of VEGF on bone is angiogenesis, but it has also been shown to encourage the proliferation and differentiation of osteoblasts[14,17]. It is because of these added properties that VEGF has been trialled in tissue engineering research along with other molecular factors and assessed on its ability to vascularise and regenerate bone[10]. VEGF is involved in many steps of healing in a fracture, including the haematoma, bone turnover and remodelling[58]. Whilst it has been tested individually it seems that VEGF functions most effectively when used at the same time as other growth factors; in particular bone morphogenetic proteins (BMPs)[10]. Aryal *et al.* recently reviewed the effect of BMP-2 and VEGF in bone tissue regeneration in fractures and it seems that although the use of BMP-2 alone has been successful it has drawbacks as it lacks the accelerated blood supply aided by the addition of VEGF[37]. The effects of VEGF and BMPs have been shown to influence each other simultaneously demonstrated when BMP antagonists were used in assessing MSC differentiation *in vivo* there was a significant decrease in VEGF production by osteoblasts, and vice versa when VEGF antibodies were used a subsequent blockade of BMP- angiogenesis occurred , indicating their corresponding roles[58]. Kumar *et al.* also demonstrated this synergistic effect in bone repair when MSCs expressing both VEGF and BMP-2 were assessed and the new bone formed for the dual-therapy group revealed significant increased peak load, toughness and stiffness of the tibial bone post fracture[29]. However the effects of dose were analysed thoroughly and they found that with higher concentrations of VEGF at a local level it has been shown to create non-functional and malformed vessels, as well as interfering with stem cell lineage when combined with MSC's at a higher dose. This results in more stem cells tending toward an endothelial lineage and reducing the amount with osteogenic effects [37].

In order to achieve functional repair of skeletal defects Gao *et al.* studied the use of MSCs in a collagen scaffold with a bolus dose of VEGF, and tested this on bone defects created in the femoral diaphysis of mice[59]. The MSC-loaded scaffold were rapidly integrated and mineralised into host bone; this was not seen in empty scaffolds and to a lesser extent in MSC scaffolds without the VEGF bolus. The results of these were further reiterated in the 2015 trial regarding the effect of VEGF-A165 on the integration of allografts in tibial defects in rabbits[60]. However, this study was done to assess the application in regard to defects other than fracture, such as those caused by infection or tumour. See below for a summary of the trials discussed here.

Type	Study	Trial design	Application	Summary
Preclinical	Kumar <i>et al.</i> (2010)[29]	<i>In vivo</i> and <i>in vitro</i>	Mouse tibia bone defects	Increased bone formation in group with BMP-2 and VEGF expressing MSCs. Increased vascularity and osteoblastogenesis compared to control. However increasing concentrations created damaged and non-functional vessels.
	Gao <i>et al.</i> (2013) [59]	<i>In vivo</i>	Rodent femur	MSC-loaded scaffolds with VEGF had increased integration and mineralisation into host bone compared to control and MSC only scaffolds.

	Ruiz-Iban <i>et al.</i> (2015) [60]	<i>In vivo</i>	Rabbit tibial defects and non-union	VEGF addition increased trabecular content and continuity, decreased failures in osteosynthesis and integration of the graft No difference found in torsional strength.
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Summary and characteristics for trials involving Vascular Endothelial Growth Factor: Table 3

4.4 Insulin like Growth Factor (IGF)

IGF's play an important role in the regulation of hormone effects, helping in osteoblast proliferation, bone resorption and also in matrix synthesis having an anabolic effect overall [17,22,61]. The group contains two proteins IGF-1 and IGF-II but the former is many times more potent and has frequently been found in fracture callus and in the expression of osteoblasts and chondrocytes during new bone formation [22]. IGF-1 levels were found to decrease initially in fracture repair but double in number 7 days post-operatively [61]. Recent trials have looked into the potential enhancement of fracture healing from the addition of IGF to standard treatments but also at the synergistic effect when added together with BMPs or MSCs. A study by Koh *et al.* looked into the differences in gene expression of IGFs and their binding proteins (IGFBPs) present in standard fractures and those with non-unions, which were created by cauterisation in rat femur fractures. RNA was extracted from the healing callus at the fracture site at various days up to 28 and analysed. They found that in the non-unions the expression of both IGF-I and II and IGFBP-6 were present in significantly higher quantities than the controls [62]. However they conclude that IGFBP-6 is generally known as an inhibitor of bone formation and therefore in opposition to the action of IGFs; more research is needed into the specific actions of the other binding proteins to see if they could help in the treatment of fracture healing. IGF-1 was tested *in vivo* with BMP-9 to assess the effect of BMP-9 induced bone formation and was found to enhance BMP-9 induction of osteogenic markers such as ALP and osteocalcin and potentiate matrix mineralisation [63]. Interestingly the exposure to the Interleukin -1B (IL-1B) had a predominantly negative effect on many growth factors and was found to induce an inhibitory migratory response from osteoblasts toward IGF-1, PDGF-BB and VEGF in normal bone [64].

In studies which focused on IGF-1 use on fracture healing, the results were positive [63,65,66]. One study investigated the effects of MSCs cultured to express IGF-1 to promote their regenerative abilities in regard to autocrine and paracrine effects on fracture healing and non-unions in mice. They concluded that the fractures with MSC-IGF improved mechanical strength and increased new bone content by speeding up mineralisation of bone. Dissected fractures from all groups were subjected to biomechanical testing and uCT analyses which measured the change in bone volume from scans taken at the beginning and those at 14 days. They found increased strength, elasticity and toughness of the callus in the group with combined MSC and IGF-1 [66]. Myers *et al.* looked at the systemic delivery of IGF-1 to enhance MSC fracture healing. This was very similar to above [65]. Kumar *et al.* reiterate the positive effects of IGF on MSCs where they tested MSC mobilisation with combinations of different growth factors and their proliferative effect, and IGF-1 was found to have 'maximum proliferative ability' of MSC *in vivo* and successful augmentation of bone [67]. See below for a table summarising trials involving IGF.

Type	Study	Trial design	Application	Summary
Pre-Clinical	Koh <i>et al.</i> (2011) [62]	<i>In vivo</i>	Rodent femur non-unions	In non-unions gene expression of IGF-11 and IGFBP-6 were significantly higher and IGFBP-5 lower.
	Chen <i>et al.</i> (2010) [63]	<i>In vitro</i>	Embryonic mouse limbs	IGF-2 enhances BMP-9 induced ALP activity and mineralisation
	Granero-molto <i>et al.</i> (2011) [66]	<i>In vivo</i> and <i>In vitro</i>	Rodent tibia fractures	Systemically transplanted MSC expressing IFG improved mechanical fracture strength and increased new bone content and mineralisation, and acted through autocrine and paracrine

				processes.
	Myers <i>et al.</i> (2012) [65]	<i>In vivo</i>	Rodent tibia fractures	In IGF-1 and MSC recipients there were increased soft and new bone tissue volumes, increased toughness and force compared to untreated or MSC alone treated mice.
	Kumar <i>et al.</i> (2012) [67]	<i>In vivo</i> and <i>In vitro</i>	Mouse tibia segmental bone defects and MSCs cultured from their bone marrow	IGF-1 had maximum proliferative ability of MSCs when testing the cells <i>in vitro</i> compared to several factors. Also when used <i>in vivo</i> for mouse tibia fractures IGF-1 use indicated a significant augmentation of bone growth and stem cell mobilisation.

Summary and characteristics for trials involving Insulin-like Growth Factor: Table 4

4.5 Transforming Growth Factor- Beta (TGF-B) and Platelet Rich Plasma (PRP)

TGF-B is involved in the proliferation of undifferentiated MSCs, osteoblast recruitment and also angiogenesis.[17] It is released by platelets when the haematomas forms and as it accumulates in bone matrix it may act as a coupling agent between formation and resorption of bone. There has been some conflicting research regarding its effects on bone, as it has been shown by a few studies to exert an inhibitory effect on osteogenic cells, while most have found it increases proliferation of these cells. Puleo *et al.* suggest that it depends on the maturation of the TGF-B cells involved at the time[22]. Although Platelet Rich Plasma (PRP) is not a cell source itself, it can be used as a source for growth factors to be added to and it can easily be isolated from fresh blood. Autologous PRP can be used in bone regeneration as it has a high concentration of platelets and therefore high levels of growth factors (such as TGF-B1, PDGF, VEGF and IGF) which enhance cell proliferation, differentiation and also are involved in chemotaxis[3].

A recent study conducted by Souza *et al.* in 2012 was about the effect of growth factors TGF-B and PDGF in osteotomy gap created in canines. They used PRP containing these growth factors to fill in the gaps created in the radius of 21 dogs. PRP is a small volume of plasma with a high concentration of platelets, and therefore also a higher concentration of growth factors that are released by platelets and other proteins [68]. The dogs were divided randomly into a control group (who had standard treatment of external fixation alone) or the experimental group (who had the fixation as well as PRP to fill the 2.00mm gap created). The results found a significant difference in the ‘median radiographic healing score’ and the ratios of healed osteotomies between the experimental and the control group at 60 days post operatively (proportion of osteotomies 4/5 healed : 1/5 healed) and concluded of the successful potential use of PRP in the future. De Gorter *et al.* also demonstrated the co-stimulation of TGF-B with another growth factor, in this case BMPs and found they further increased expression of osteoblast-specific genes and ALP activity compared to BMPs alone[69] and in an experiment of TGF-B1 and demineralised bone matrix in local application of osteotomies in dogs, increased collagen and proteolytic ability was found[70].

In order to maximise the effects of PRP, the delivery of it along with growth factors and stem cells has also been looked at in recent years, and ‘chitosans’ have been trialled to provide a more vascularised scaffold in bone healing; the results look positive as chitosan-PRP incorporated into a bone scaffold highly induced MSC differentiation[71]. Similarly PRP has been combined with calcium phosphate cement in different ratios to assess its properties. It was found that osteoregeneration, PDGF and other growth factor release and ALP activity were all increased in the higher concentrations of PRP (10 and 15 wt%) with the cement, and had a significantly better affect than other groups *in vitro*. In general PRP-CPC was found to be a stable scaffold and after immersion in simulated body fluid for 32 days PRP was retained in the cement matrix[72]. In a study of minimally invasive intervention (MII) of delayed or non-union fractures 24 patients underwent treatment, with

some have MII with iliac crest bone marrow aspirate and blood (containing MSCs and PRP) injected into the fracture site. No complications occurred in either group and the median time to union was 3 months for the control, and 1.5 months for the experimental group. However, they do explain that both results were significantly faster than expected from similar fractures[73].

However, the use of PRP in skeletal defect has been shown to have no effect on bone healing by Peerbooms *et al.* At one week postoperatively from tibial osteotomies the bone density was significantly lower in the PRP group than the control, and this was demonstrated again at 12 weeks, although at 6 weeks there was no significant difference[74]. Additionally Leukocyte-PRP was evaluated in autografts of bone defects produced in rabbits and bone matrix was found to be significantly less in the defects treated with L-PRP compared to just an autograft. It was thought to have interfered with signalling of TGF-B1 and other pathways in maintenance of stem cells[75]. PRP was also tested on human synovium-derived MSCs and was seen to have an overall negative effect on cell differentiation[76].

The main challenge presenting with TGF-B1 use is due to a short half-life, but it has been found that when combined with a novel vector in a particular composite for a scaffold it greatly accelerated bone healing in segmental defects and seemed to maintain its' bioactivity[77]. See below for tables summarising the trials discussed involving TGF-B and PRP.

Type	Study	Trial Design	Application	Summary
Pre-Clinical	Souza <i>et al.</i> (2012) [68]	<i>In vivo</i>	Canine Radial ostectomy	PRP (containing TGF-B and PDGF) group radiographic healing score increased significantly from 0-60 days, and proportion of healed ostectomies was much higher than the control
	De Gorter <i>et al.</i> (2011)[69]	<i>Ex vivo</i>	Mouse pluripotent MSCs	Co-stimulation of BMPs and TGF-B increased expression of osteoblasts, ALP activity and mineralisation compared with BMPs alone.
	Servin-trujillo <i>et al.</i> (2011) [70]	<i>In vivo</i>	Canine tibia, open osteotomy	Improvement and restoration of bone in graft with TGF-B1 and early formation of bone callus and bone regeneration compared to controls. There was also increased collagen and proteolytic activity but no changes in ALP and clinical parameters.
	Pan <i>et al.</i> (2014) [77]	<i>Ex vivo</i> and <i>In vivo</i>	Rabbit derived MSCs and rabbit long bone defects	<i>Ex vivo</i> the group with MSCs and TGF-B1 had significantly higher type I collagen, osteocalcin, osteopontin and ALP markers compared to other groups with MSCs alone. This group had accelerated bone regeneration when applied to rabbit bone defects <i>in vivo</i> . Conclusions were based on X-rays, histology and biomechanical exams.

Summary and characteristics for trials involving Transforming Growth Factor- Beta: Table 5

Type	Study	Trial Design	Application	Summary
Pre-Clinical	Souza <i>et al.</i> (2012) [68]	<i>In vivo</i>	Canine Radial ostectomy	PRP (containing TGF-B and PDGF) group radiographic healing score increased significantly from 0-60 days, and proportion of healed ostectomies was much higher than the control
	Ko <i>et al.</i> (2013) [72]	<i>In vivo</i> and <i>In vitro</i>	Rabbit Femurs	Growth factor release and ALP had significantly better effect on 10 and 15 wt% (higher PRP conc) than on other groups when mixed with calcium phosphate bone cement (CPC). PRP was still retained in cement matrix after 32 days immersion <i>In vivo</i> osteoregeneration was increased in the PRP-additive group, with this group showing earlier breakdown of bulk dense implants compared to CPC-only group.
	Peerbooms	<i>In vivo</i>	Human	Bone density was significantly lower in the PRP group

	<i>et al.</i> (2012)[74]		skeletal defects	compared to control at 1 and 12 weeks in wedge fracture. Overall patients did not benefit from PRP addition in this procedure.
	Giovanini <i>et al.</i> (2013) [75]	<i>In vivo</i>	Rabbit skull defects	L-PRP treated defects had significantly less bone matrix than control. Results suggested that L-PRP induces a cross-reaction between TGF-B1 and other factors, impairing the osteoconductive properties of the autograft.
	Lee <i>et al.</i> (2014) [76]	<i>Ex vivo</i>	Human synovium derived MSCs	PRP on these cells had an overall negative effect and does not induce stem cell differentiation.
Clinical	Calori <i>et al.</i> (2013) [57]	<i>In vivo</i>	Human bone non-unions	RhBMP-7 vs PRP: clinical and radiological union in 87% of rhBMP-7 compared to 68% in PRP, and a lower clinical and radiographical healing time for the BMP-7 group (3.5 vs 4 months, and 8 vs 9 months), however this study does demonstrate that there was some success in using PRP.

Summary and characteristics for trials involving Platelet Rich Plasma: Table 6

4.6 Platelet Derived Growth Factor (PDGF)

PDGF is a signalling molecule which plays a role as a ‘mitogen’ to stimulate mitosis and increases the number of bone producing cells and along with other growth factors plays a role in angiogenesis[78]. Caplan *et al.* wrote a review of the effects observed about PDGF with MSCs on bone regeneration and they conclude that in bone repair PDGF takes on the role of mobilising pericytes associated with vessel formation and the release of activated MSCs, providing stronger healing and bone or callus formation[79]. PDGF is released from platelets and can induce the differentiation of MSCs into many different cell types including osteoblasts and fibroblasts. It has been shown in preclinical studies that recombinant PDGF-BB enhances bone repair, but the subjects for this study had compromised healing such as diabetes and osteoporosis[80]. Tan *et al.* found that platelet derived factors expanded MSCs *ex vivo* and influenced their response *in vivo*, as their increase correlated with boosted response of MSCs and were much higher in patients who had PRP injected into the iliac crest [81]. See below for a summary of trials discussed here.

Type	Study	Design	Application	Summary
Pre-Clinical	Souza <i>et al.</i> (2012) [68]	<i>In vivo</i>	Canine Radial ostectomy	PRP (containing TGF-B and PDGF) group radiographic healing score increased significantly from 0-60 days, and proportion of healed ostectomies was much higher than the control
Clinical	Tan <i>et al.</i> (2015) [81]	<i>In vivo</i>	Human bone marrow in fracture patients	Direct positive correlation between changes in bone marrow MSCs and changes in serum PDGF, so they seem to influence MSC response in fracture patients.

Summary and characteristics for trials involving Platelet Derived Growth Factor: Table 7

4.7 Fibroblast Growth Factor (FGF)

In this family of growth factors FGF-1 and FGF-2 (basic FGF or b-FGF) have been studied the most, and have been identified in the early stages of fracture healing with an important regulatory role in bone repair, including angiogenesis [22]. *In vivo* bFGF has been found to help maintain the osteogenic qualities of bone marrow MSCs[32]. It was found that DJ-1 (new angiogenic factor secreted by MSCs) promotes angiogenesis by activating the FGF-1 signalling and enhanced bone regeneration in a rodent model of fracture repair[82].

However in a study by Biver *et al.* they found a treatment-duration dependant inhibitory effect of FGF-2 on mineralisation in bone, regardless of the initial increase in cell proliferation seen and it was found to inhibit the up-regulation of BMPs as FGF-2 completely blocked the increase of BMP2 and BMP-4[83].

See below for a summary of trials involving FGF

Type	Study	Design	Application	Summary
Pre-Clinical	Kim <i>et al.</i> (2012) [82]	<i>In vivo</i> and <i>In vitro</i>	Rodent fracture	DJ-1 was shown to enhance bone regeneration and stimulate blood vessels and new bones through activation of FGF-1
	Biver <i>et al.</i> (2012) [83]	<i>Ex vivo</i>	Human Mesenchymal Stem cells	FGF2 inhibited MSCs differentiation and upregulation of BMPs.

Summary and characteristics for trials involving Fibroblast Growth Factor: Table 8

5. Limitations

There are several limitations that apply to this review. Firstly due to accessibility issues with relevant papers and inability to fully analyse all valid database search papers with their reference lists we may have missed some relevant material and this increases the effect of selection bias. Similarly there may have been important information published in languages not included in our search so this could influence my overall conclusions drawn. Therefore we may have missed some negative results in terms of MSC and growth factor use in fractures and non-unions. However we tried to keep my inclusion criteria broad in not limiting the publication year, of the type of trials looked at, as well as hand searching for related topics to broaden my review from database searches and we tried to be as thorough as possible.

Another limitation lies with the variety of papers and results we have included, in terms of my quantitative data, which doesn't allow for accurate comparison amongst the studies found and only loose conclusions formed for each growth factor. Also my search results yielded many more papers regarding MSCs or BMPs but less so with the other growth factors, which tend to have only been reviewed in the last few years. Therefore our review does focus predominantly on the use of stem cells and bone morphogenetic proteins over other factors. There were many more pre-clinical trials found than clinical trials as well so it is hard to compare or draw conclusions for the outcomes seen in pre-clinical studies with potential effects if similar trials were conducted on human participants.

6. Conclusion

In conclusion, it seems that there are many positive findings related to the use of mesenchymal stem cells and various growth factors in fracture healing and the treatment of non-union. MSC use in alternative grafts seem to be successful and although not many trials exist, the use of alternative sourced MSCs other than bone marrow and the delivery via a systemic application provide novel ways for broadening the potential benefits of MSC in fracture repair. Alternative MSCs such as adipose cells may overcome previous drawbacks of bone marrow MSCs such as lack of vascularisation in bone repair and the addition of other factors to MSCs was also successful in enhancing their effects, particularly BMPs and VEGF.

Recombinant BMP-2 and BMP-7 have been thoroughly analysed and the majority of studies have found them to be significantly beneficial in non-unions or delayed fractures and in accelerating healing clinically. But recent studies have also highlighted the potential use of BMP1-3 and BMP-9 in bone repair and the novel carrier device OSTEOGROW with BMP-6. The 'diamond concept' draws together the synergistic effects of growth

factors, scaffolds and MSCs and has so far proved to be successful. Further clinical studies are needed to confirm the definitive benefit in the treatment of non-unions. However, the results so far are very positive.

VEGF has been demonstrated to be beneficial for angiogenesis and has an enhanced effect when used in conjunction with other factors. IGF has had mixed reviews on its action in bone repair but so far results in fracture healing are positive and especially useful when expressed by MSCs. TGF-B1 and PRP have been shown to be beneficial in fracture healing but there are diverse results regarding the benefit of PRP. FGF has been shown to be involved in vital pathways for bone regeneration but there are only a few studies on this growth factor and more are needed pre-clinically. Overall, the study of these growth factors is still in the early stages, with more pre-clinical research available than clinical. Much more research is needed on each of these before testing the potential applications in a clinical trial or for therapeutic applications. However, so far the results of these studies seem very promising for the future of bone regeneration and the potential use of growth factors and mesenchymal stem cells.

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