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REVIEW

Efficacy of minimally invasive techniques for enhancement of fracture healing: evidence today

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Abstract The successful treatment of nonunions represents a major challenge for orthopaedic surgeons. Lately, ongoing advances made in the field of molecular medicine and molecular biology have increased our understanding of the pathways and involvement of mediators surrounding the bone healing process. As a result, the surgeon's armamentarium has been increased in terms of options for intervention. This article aims to provide an overview of minimally invasive techniques applicable in the treatment of nonunions of fractures.

Introduction

The incidence of impaired healing of long bone fractures has been reported to range between 5 and 10% [25, 88, 94, 118, 119]. It requires complex and expensive treatment and a variable degree of morbidity is often a common finding. Nonunion represents a challenging clinical problem for both the patient and the treating physician [10]. The implementation of treatment protocols can be time consuming. Not infrequently, nonunion is associated with chronic pain, functional and psychosocial disability, to say nothing of the socio-economic burden [27, 116].

Implementation of a treatment strategy for aseptic nonunions depends on an accurate assessment and classification of the nonunion [52, 73]. Apart from the physiology of the host (patient) both the presence of mechanical stability and the state of the surrounding soft tissue envelope are of paramount importance.

Autologous cancellous bone grafting (ABG) remains the gold standard biological method used to promote bone healing by stimulating the local micro-environment at the nonunion site [7, 9, 34, 35, 87, 101]. However, its limited availability, as well as the donor site morbidity and complications such as chronic pain, neurovascular injury and infection have dictated the need for the development of alternative methods of biological stimulation [3, 15, 17, 24, 34, 35, 39, 49, 96, 105, 112]. Other methods of biological stimulation, used either alone or in combination, include allo-grafting, the use of electrical, ultrasound, and shockwave stimulation, a variety of bone graft substitutes, with either osteoconductive or both osteoconductive and osteoinductive properties and bone marrow injections [26, 52, 73, 84, 87, 90, 122].

Assuming that the mechanical stability factor is sound, each treatment option of biological stimulation may require a degree of surgical dissection and exposure for the delivery of bone stimulating substances or autologous bone graft. Extensile surgical exposures ideally should be avoided as they could negatively affect the already compromised local biological environment of the nonunion site. In this context, where appropriate, minimally invasive techniques of biological stimulation are desirable (Fig. 1).

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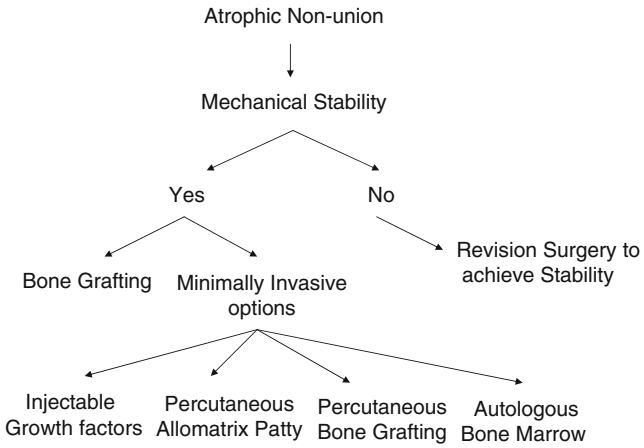

Fig. 1 Considerations for the treatment of atrophic nonunions

The purpose of this study therefore was to investigate the current advances in the enhancement of the fracture healing response and treatment of nonunions with minimally invasive techniques.

Materials and methods

We searched MEDLINE for general keywords such as 'nonunion', 'autologous bone marrow', and 'minimally invasive techniques', both isolated or in combination with specific words including 'growth factors', 'bone marrow injection' and 'percutaneous patty', from 1970 until the present. For paper selection, the initial inclusion criteria were studies reporting results on minimally invasive techniques applied in the treatment of fresh fractures and nonunions both in humans and animal models. We focussed our research methodology retrieving studies on four potential options on biological stimulation using non-invasive techniques: (1) injection of growth factors, (2) percutaneous bone grafting, (3) percutaneous patty and (4) autologous bone marrow injections. Such parameters as the type of nonunion, number of treated fractures, time to union, and technique used were extracted and analysed from the studies. The reference list of studies which met the inclusion criteria were further screened for inclusion of manuscripts which could have been omitted from the initial screening process.

Exclusion criteria included publications in the non-English language or studies having incomplete documentation.

Results

A total of 99 articles met the inclusion criteria [1–6, 11–13, 16–22, 25, 28–32, 36, 37, 39–51, 53–72, 74–83, 85, 86, 88, 89, 91–93, 95–100, 102–113, 115–117, 119–121, 123].

Studies selected were grouped as experimental or clinical as described in this paper.

Experimental studies

Injection of growth factors for enhancement of fracture healing

The usefulness of this technique is still under intense study by several investigators using different animal models [4, 67, 77, 95, 97, 100]. Simple injection of osteoinductive growth factors in subcutaneous tissue has showed that it can promote bone formation [97, 100]. A simple local percutaneous injection in fractures has been shown to enhance bone healing [23]. Similarly, injection of BMP-2 in a rat segmental defect model showed a dose-dependent effect, with a low dose resulting in large amounts of fibrous tissue and cartilage while larger doses resulted in trabecular bone and small amounts of cartilage [4]. Such a protein-based approach though poses potential disadvantages derived from the short protein half-life and the poor retention in the fracture, nonunion or defect site. Gene transfer could overcome these limitations. Several viral vectors carrying BMP-2 cDNA have been studied on animal models over the years [4, 5, 104]. All showed promising results for future clinical application.

The potential of other molecules have been studied alongside BMPs in recent years. Basic fibroblast growth factor (bFGF) injection was found to contribute to the formation of a larger cartilaginous callus but without any accelerating effect in fracture consolidation [77]. Rozen et al. studied the effect of enhancement of each stage of fracture healing by injecting IL-6 during the inflammatory stage, PTH 1-34 during the granulation stage and PTH 28-48 during the callus formatting stage [95]. Their findings revealed a 300% increase in mechanical resistance in the treated rat tibiae [95].

Percutaneous patty administration

The inspiration for the development of an injectable osteoinductive graft for the treatment of bone defects and nonunions came with the use of demineralised bone matrix (DBM). So far, the available animal studies prove that this technique can be used in such pathologies [16, 109, 110]. Comparison with autogenous bone grafting in animals has shown that injectable allomatrix patty was as effective as autograft bone, and was also excellent in handling [107]. DBM, when applied in nonunions, tend to remodel from the periphery, which may explain a lower volume of bone but without any difference in bone mineral density and signs of radiographic or histological healing [54]. In a canine nonunion model, DBM stimulated defect

healing, but its combination with bone marrow exerted a greater healing potential [108].

Bone marrow injection for fresh fractures and nonunions

The use of BM to facilitate the healing of bone defects and nonunions is extensively covered by several animal models. The findings reported have been favourable [21, 83]. In segmental bone defects of canine ulna the animals treated with autologous bone marrow united in contrast with the untreated control animals which developed nonunions [42]. Percutaneous BM injections were also able to heal bone defects in rabbits [61]. In dogs suffering from tibial shaft nonunions the injection of BM or demineralised bone matrix in the site of nonunions revealed that both were able to stimulate the healing of the defect [110]. The combination though appeared to be more potent compared to each of them alone. BM combined with platelet-rich plasma and freeze-dried bone allografts could serve as an alternative treatment modality [21].

Ma et al. reported a new method of promoting fracture healing by the administration of multiple cryopreserved injections of BM. In a rabbit model such an approach increased the incidence of union, the radiological bone volume and the bone mineral density [63].

Clinical studies

Injection of growth factors for treatment of nonunions

The injection of growth factors at the nonunion site could be used as an alternative, less invasive technique. The rationale behind this method may be associated with signal transduction upregulating the activity of host mesenchymal stem cells, with the increase of vascularity but also with the enhancement of the low levels of expression of regulatory molecules encountered at the cells at the nonunion site [78]. Currently, BMP-2 and BMP-7 are commercially available and the results obtained by open implantation of these molecules at the nonunion site appears to be promising [2, 11, 28, 29, 36, 37, 40, 52, 54, 98, 111, 121]. However, as far as we know, there are no studies on the outcome of this technique in humans. This is mainly due to formulation problems and the lack of a perfect delivery vehicle [97].

Percutaneous bone grafting

Minimally invasive bone grafting for tibial nonunions could serve as an alternative to open reduction with favourable results. The technique consists of a cylindrical cut through the nonunion site, replaced by a similar piece obtained from the iliac crest. Kettumen et al. evaluated this technique in 41 patients, achieving union in 37 (90%) by a period of 13 weeks (range 10–48). Similarly, Bhan and Mehara [5] and Maghsudi et al. [64] reported union rates in tibial nonunions of 86% (18 out of 21 patients) and 88% (seven out of eight patients), respectively. Maneerit et al., in a randomised prospective trial, compared the results of open reduction and percutaneous bone grafting for tibial fractures. They reported equal rates of union by both techniques, but with definite superiority of the percutaneous technique in terms of blood loss and reduction of operative time [68]. Alternatively, the technique of arthroscopy could be applied for the visualisation, exposure and application of bone grafts at the nonunion site. Johnson et al. successfully treated eight patients suffering from nonunions by arthroscopic delivery of cancellous bone grafts [51].

Percutaneous patty

Wilkins et al. used injectable allomatrix patty for the treatment of 35 patients suffering from long bone nonunions [116]. Their results revealed an 85.1% union rate. On the other hand, a recent study by Ziran et al., which evaluated an open application of a new calcium sulphate-demineralised bone matrix/allomatrix in the treatment of nonunions, presented an unacceptably high rate of complications which included postoperative drainage (54%), deep infection (34%) and an overall failure rate of 46% [123].

Bone marrow injection

The osteogenic properties of BM were first reported by Gourjon et al. in 1869 after the observation that injections of bone marrow to heterotopic sites resulted in the formation of new bone. Since then several reports have documented the osteogenic potential of BM which was attributed to primitive wandering cells capable of osteogenic differentiation. Our understanding of these observations was improved in 1971 with the discovery of "bone marrow derived osteogenic precursor cells" or the so-called BM-derived mesenchymal stem cells.

Mesenchymal stem cells (MSCs) are osteoprogenitor cells, readily available to differentiate towards chondrocytes or osteoblasts and support endochondral and intramembranous ossification. The frequency of MSCs obtained from BM aspirates is about 0.01% or lower [20]. Hernigou et al. concentrated BM by centrifugation, preparing injections containing an average of 2,579±1,121 progenitors/ml compared to the 612±134 progenitors/ml in simple bone marrow aspirates [47]. However, it should be clear that bone itself, as well as many other tissues like periosteum, fat and muscle host mesenchymal stem cells capable of producing bone [91]. Furthermore, it was

suggested that genetic reprogramming or transdifferentiation can occur, so fully differentiated cells of one type could switch into another fully functional cell type [20]. These issues though, are poorly understood, and currently there is no indication for the existence of the "ideal progenitor cell" responsible for fracture healing.

In any case, the fate of MSCs is influenced by the local microenvironment, chemotaxis and interactions with the extracellular matrix [70, 82]. During fracture healing they can migrate outside BM and differentiate into osteoblasts to form callus [106]. It was also shown that they could also contribute to fracture healing after systemic injection, being localized at the fracture site [22].

The concept of the delivery of fresh autologous bone marrow as an autograft has developed over the last two decades. It is an inexpensive method that requires minimal hospitalisation and patient care. Patients undergoing BM harvesting can return to their daily activities within 12 hours [80]. Several animal models together have proven the feasibility of this technique both in enhancement of the outcome in fresh fractures and in the treatment of delayed unions or nonunions.

For fresh fractures The feasibility of BM injection in fresh fractures with high risk for nonunions is currently under investigation. Khanal et al., in a prospective randomised study of tibial fresh closed fractures, compared the results obtained by conventional treatment and injection of 15 ml of bone marrow [57]. In the group that received BM all fractures united, while in the control group the union rate was 95% (19 out of 20). Patients who received BM injection experienced a lower time to union. Another application of BM was in a case of non-ossifying fibroma of a radius of a ten-year-old girl with a pathological fracture, whereby the curettage and the concomitant filling of the void with autologous bone marrow resulted in complete union in 12 months [44].

For nonunions The first application of injectable bone marrow for un-united fractures appeared in the 1980s [45, 50]. Connolly et al., using relatively large amounts of bone marrow of 100–150 ml, successfully treated eight out of ten tibial nonunions [18]. Healey et al. evaluated the feasibility of this technique in eight patients suffering from nonunion after reconstruction for primary sarcoma. Five of the eight patients received chemotherapy. The authors reported union in five cases (three out of five of whom received chemotherapy) [45]. Thereafter, bone marrow injections were widely used for tibial [18, 19, 32, 99, 103, 111, 113], femoral [69, 103, 115], humeral [32, 102, 103, 115], and clavicular nonunions [107], and also for nonunions of radius and ulna [32, 101, 103]. Various amounts of bone marrow were used, from 10 ml to 150 ml, without showing any difference in the success rates (Table 1). However, a definite relationship was apparent between the number of osteoprogenitors injected and the rate of union [47]. Hernigou et al. compared the number of mesenchymal stem cells injected in 53 cases of successful union to seven cases where this technique failed [47]. The united cases received 54,962 ± 17,431 MSCs while the unsuccessful ones received 19,324 ± 6843 MSCs [47].

In total, 336 cases of nonunions have been described. From those, 249 (72.1%) were tibial nonunions, 40 (11.6%) were femoral nonunions, 20 (5.8%) were humeral and the rest (36; 10.4%) were nonunions of the radius and ulna (Table 1). The mean age was 38.1 years. The union rate after treatment with percutaneous injection of autologous bone marrow was 88.8% (296 out of 336 nonunions; range 57%–94%). The mean time to union was 4.8 months (range 2.5–8.1).

In addition to pure bone marrow grafting, composites of allogenic demineralised bone matrix impregnated with aspirated bone marrow were evaluated for their efficacy in nonunions of long bones. This approach resulted in an overall success rate of 88% [117]. Similarly, Kocialkowski et al. reported the successful treatment of 11 cases (100% success rate) of nonunions with the use of composites of 1:1 collagen and ceramic enriched with bone marrow [59].

For other orthopaedic applications The value of bone marrow is not only restricted to the enhancement of fracture union and healing of nonunions, but rather has proved important in other orthopaedic pathologies (Fig. 2). The isolation of osteoprogenitors contained in BM, followed by their uploading onto scaffolds and implantation in bone defects and difficult nonunions, is another successful method of treatment [81, 93]. Orozco et al. has proposed a method comprising aspiration of 7–30 ml of BM at 12 days prior to surgery, followed by mesenchymal stem cell isolation and expansion [81]. During surgical fixation the cultured cells were mixed with β-tricalcium phosphate matrix and placed at the nonunion site. Radiological callus formation occurred in five out of six patients at an average of eight weeks (range 6–24 weeks). Additionally, culture-expanded mesenchymal stem cells are used in distraction osteogenesis showing acceleration of regeneration with shortening of the treatment time [58]. Another application of BM consists of BM stem cell auto-transplantation at the ischaemic lower limb, which resulted in an improvement of the peripheral blood flow and percutaneous oxygen partial pressure [80]. Moreover, BM and its osteoprogenitors were used in spinal fusion [22, 73], osteonecrosis of the femoral head [30, 31, 46], bone cysts [14], osteochondral defects [13], tendon osteointegration [62], and in facilitation of nerve repair [12, 120].

Table 1 The effectiveness of bone marrow (BM) injections for nonunions

First author/year	Number of nonunions	Type	Mean age (y), *n* (range)	United, *n* (%)	Time to union (months), *n* (range)	Comments
Healey/1990 [45]	8	Femur 8	27 (6–60)	5 (62.5)	4.95 (1–9)	Nonunion occurred after reconstruction for lower limb cancer resection
						50 ml percutaneous injection
Connolly/1991 [18]	10	Tibia 10	30 (18–82)	8 (80)	6.7 (5–10)	100–150 ml of BM aspirated and injected
						Fractures were immobilised by cast
Garg/1993 [32]	20	Tibia 15 Humerus 3	35 (18–65)	17 (85)	5 (3–7)	Percutaneous injection of 15–20 ml of BM
		Ulna 2				
Sim/1993 [102]	11	Tibia 8 Femur 1	38 (19–62)	9 (81)	2.5 (1–5.75)	Percutaneous injection of 50–200 ml of BM
		Humerus 1				
		Ulna 1				
Garg/1995 [33]	Case report	Tibia	12	1 (100)	18	~20 ml of BM were aspirated and injected in pseudoarthrosis while limb was placed in cast
						After 3 weeks the procedure was repeated
Matsuda/1998 [69]	7	Femur	53 (24–70)	4 (57)	na (5–9)	Percutaneous technique with injection of 150 ml of BM
						Two infections encountered resulting in failed union
Sebecic/1999 [99]	Case report	Tibia 1	44	1 (100)	5	150 ml injected and immobilisation achieved by external fixation
Siwach/2001 [103]	72	Tibia 42	41.2 (26–56)	68 (94)	na	A maximum of 30 ml of BM aspirated and injected percutaneously at nonunion site
		Femur 8 Humerus 12				The procedure was repeated where required after 4–6 weeks after initial injection
		Forearm 10				
Wang/2001 [113]	56	Tibia	na (19–72)	53 (94)	na	BM aspirated and injected percutaneously at nonunion site
						The procedure was repeated every month 2–3 times
Wilkins/2003 [115]	69	Tibia 36 Femur 16	42 (15–81)	61 (88)	8.1 (2–36)	Percutaneous technique with BM combined with allograft demineralised bone matrix
		Humerus 4				
		Others 13				
Goel/2005 [38]	20	Tibia 20	37.5 (24–60)	15 (75)	3.5 (1.5–5.5)	A maximum of 15 ml of BM was aspirated and percutaneously injected
Hernigou/2005 [47]	60	Tibia 60	40 (18–78)	53 (88)	3 (1–4)	An average of 306±24 ml was aspirated, concentrated to 20 ml and percutaneously injected
Tetreault/2007 [107]	Case report	Clavicle	39	1 (100)	na (within 12)	Percutaneous injection of 10 ml of BM

na not available

Level of Evidence: All presented studies are 2C except case reports which are 1C

Discussion

The normal physiological reaction to fracture is a spontaneous sequence of events briefly summarised as initial inflammation, followed by soft and hard callus formation and ultimately bone remodelling [91]. When this process does not occur, as in cases of nonunions or traumatic bone defects, surgical intervention is required and is commonly combined with autologous grafting, which enhances the local environment in terms of osteoprogenitor cells, structural substrate and bone inducing proteins [92].

The surgical technique usually implies extended incisions for the revision of fixation of the affected bone [3, 17, 39, 49, 65, 96, 105, 112]. The use of judiciously applied orthopaedic

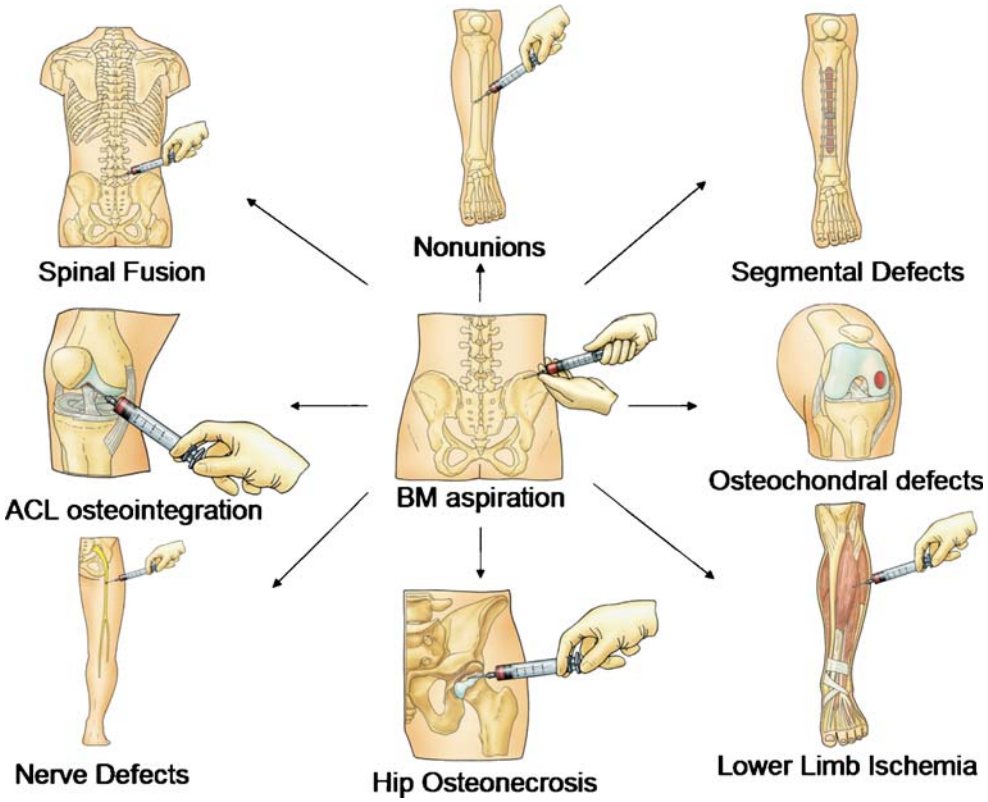

Fig. 2 Bone marrow applications

implants through limited incisions to treat fractures and nonunions is a recently developed alternative to the traditional techniques [92]. Similarly, the introduction of osteoprogenitor cells or osteoinductive materials has gained more support lately with satisfactory results [20, 45, 47, 71].

Injection of growth factors is based on the tremendous effect that these molecules exert to induce upregulation of bone healing [91]. Currently, a variety of molecules including BMP-2, BMP-7, platelet-derived growth factor, fibroblast derived growth factor and PTH have been used for the treatment of nonunions in experimental studies (animal models) [4, 7, 8, 50, 67, 71, 77, 95, 97, 100, 114]. The results have shown that the molecules are capable of upregulating the healing process, thus accelerating the fracture consolidation and mechanical properties [50, 71, 77, 89, 95, 100, 114]. However, there are no clinical data involving injectable formulations. This can be attributed to the slow development of appropriate delivery vehicles (liquid formulations), the lack of identification of the ideal concentration and the lack of safety data.

Percutaneous bone grafting could be the alternative to local application of osteoinductive molecules. In animal models, it has been well established that DBM is capable of stimulating new bone formation, but also that its combination with osteoprogenitor cells produces better results [16, 109, 110]. In humans this approach proved controversial as the use of injectable demineralised bone resulted in a high rate of complications [123]. Percutaneous bone grafting on the other hand has been proven successful in the safe treatment of nonunions [5, 54, 56]. This could be attributed to the fact that autologous bone chips contain the required structural osteoconductive substrate combined with the osteoinductive properties of the autologous growth factors and osteoproductive cells. Although this has been proven to be true, donor site morbidity, potential unavailability and restrictions due to the size of the defect are limiting factors to be taken into account.

Percutaneous bone marrow implantation for the treatment of nonunions is a low risk inexpensive procedure with high biological activity [79]. The results from published papers indicate that the success rate of treating established nonunions with percutaneous injection of autologous bone marrow can be as high as 88.8% (Table 1). Literature describing conventional approaches using autologous bone grafts have reported a variable success rate. Tibial nonunions heal at a rate of 90–95% [25, 88, 119], whereas in femoral nonunions the rate of healing is slightly lower, ranging from 78.3% to 92.3% [43, 85, 86, 117]. Similarly, in humeral nonunions the healing rates vary between 76% and 100% [1, 41, 48, 72]. Therefore, it can be clearly concluded that percutaneous bone marrow injection could be as effective as open autologous grafting. This efficacy can be attributed to the implantation of viable osteoprogenitor cells. However, the concentration of MSCs may vary significantly between individuals, aspiration sites, and aspiration technique [66, 74, 75].

Over the past several decades, minimally invasive surgery has revolutionised many fields of medicine. In orthopaedics and especially in challenging situations, it has decreased the damage to soft tissues, minimised scaring, reduced postoperative pain and improved recovery time [51, 68]. All of these have lead to accelerated return to work, sports and normal daily living. Among these techniques the choice of the most appropriate approach can be difficult. The injection of growth factors can enhance the local biology and optimise the potential of osteoprogenitors. This technique though is not fully developed for human use yet and will not benefit poor local environment [97]. Percutaneous bone grafting seems to be an excellent alternative to extensive open bone grafting. It enhances the local environment with fresh bone and cells required to facilitate healing [5, 56, 64]. The disadvantages of this technique can be the limited extent of grafted bone, the potentially small number of grafted osteoprogenitors, and the small but present donor site morbidity. Autologous bone marrow injection on the other hand, augments cellular numbers and has extremely low donor site morbidity but stimulates the grafted cells to produce a structural osteoconductive substrate [79]. Our view is that all these techniques can be used in the treatment of simple or challenging cases with favourable results. However, simple cost-effective techniques such as autologous bone marrow injection have been proven to have results comparable to those of open techniques. By standardised nomenclature, enhanced training, and rigorous evidence-based research, these emerging techniques will continue to improve the surgical outcomes for hundreds of thousands of patients.

Conclusion

Minimally invasive techniques for the treatment of non-unions and enhancement of fracture healing appear to be effective as a single treatment modality. Current literature, although limited, supports the idea that a simple injection of bone marrow can have the same results as those obtained from open techniques. However, more studies and randomised trials are needed for definitive conclusions.

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