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REVIEW ARTICLE

The role of stem cells in fracture healing and nonunion

Hangama C. Fayaz · Peter V. Giannoudis · Mark S. Vrahas · Raymond Malcolm Smith · Christopher Moran · Hans Christoph Pape · Christian Krettek · Jesse B. Jupiter

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Abstract Nonunion and large bone defects present a therapeutic challenge to the surgeon and are often associated with significant morbidity. These defects are expensive to both the health care system and society. However, several surgical procedures have been developed to maximise patient satisfaction and minimise health-care-associated and socioeconomic costs. Integrating recent evidence into the diamond concept leads to one simple conclusion that not only provides us with answers to the "open questions" but also simplifies our entire understanding of bone healing. It has been shown that a combination of neo-osteogenesis and neovascularisation will restore tissue deficits, and that the optimal approach includes a biomaterial scaffold, cell biology techniques, a growth factor and optimisation of the mechanical environment. Further prospective, controlled, randomised clinical studies will determine the effectiveness and economic benefits of treatment with mesenchymal stem cells, not in comparison to other conventional surgical approaches but in direct conjunction with them.

Introduction

One hundred and thirty years ago, the German pathologist Cohnheim reported the existence of nonhaematopoietic stem cells in bone marrow. He injected an insoluble aniline dye into the veins of animals and observed the appearance of dye-containing cells in wounds he shaped at a distal site. He indicated that most cells originated from the blood-

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stream, i.e. from bone morrow. His publication initiated the concept of bone marrow as the source of fibroblasts that build collagen fibers in wound healing [5]. Recently, the potential benefits of human mesenchymal stem cells (MSCs) have received increasing attention in a wide variety of biomedical fields [25, 38, 41]. However, researchers often report studies of MSCs involving different methods of isolation and growth as well as different approaches to describe the cells. Thus, it is increasingly difficult to evaluate and compare study outcomes. Also, the term mesenchymal stem cell has not been well defined. The literature associates this term with multiple properties, including self-renewal, tissue repair and differentiation into other specialised cell types, such as bone, cartilage, adipose and muscle cells. MSC are defined as nonhaematopoetic stromal cells that contain multilineage differentiation ability and are able to stimulate the growth of bone, cartilage, adipose tissue, tendon and muscle [43]. To build a more uniform description of MSCs, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has proposed minimal criteria to define human MSCs [modified from 9]:

- MSC must be plastic-adherent
- MSC must express CD105, CD73 and CD90
- MSC lack expression of CD45, CD34, CD14, CD11b, CD79a, CD19 and HLA-DR surface molecules
- MSC must differentiate to osteoblasts, adipocytes and chondroblasts in vitro
- MSC lack of expression of haematopoietic Antigen

Using bone-marrow-derived MSCs to repair injured tissue is a complex, multistep procedure that includes mobilising, homing and reparative interventions. Once specific signals are released from injured tissue, MSCs are stimulated to leave their niche and circulate (mobilisation). Mobilisation is followed by arrest of the circulating MSCs within the vasculature of the tissue and transmigration across the endothelium (homing). Finally, MSCs proliferate and differentiate into mature cells [32]. As indicated by Honczarenko et al., cytokines and chemokines play a major role in managing the mobilisation, trafficking and homing of progenitor cells; MSCs convey a particular set of chemokine receptors, such as CCR1, CCR7, CCR9, and CXCR4-6. Chemokines (e.g., CXCL12) that are bound by these surface receptors initiate cellular response-specific chemotaxis events and ß-actin filament reorganisation. CXCL12 is of paramount importance in bone marrow MSC homing and localisation within the bone marrow [23]. Current research focuses on strengthening the natural reparative ability of the body by delivering MSCs formed from a patient's own tissues to the site of injury.

Nonunion

Despite advanced and optimised surgical procedures, approximately 5–10% of the 7.9 million fractures sustained annually in the United States fail to achieve bony union [16]. According to the US Federal Drug Administration (FDA) council, nonunion is defined as a fracture for which a minimum of nine months has elapsed since the injury and for which there have been no signs of healing for three months. However, as pointed out by Russell et al. and Taylor et al., it is difficult to set a fixed time period in the definition of nonunion [46, 50]. Taylor et al. indicated that the nine months since injury criterion cannot be used for every fracture and recommended that fracture nonunion of long bones be recognised after a minimum of six months without any improvement toward union [46, 50]. Based on radiographic appearance, aseptic nonunions are categorised as atrophic or hypertrophic [37]. Whereas atrophic nonunion involves poor vascularity at the fracture site and demonstrates little callus formation, the therapeutic management of atrophic nonunion entails decortication, bone grafting and stabilisation. Thorough removal of the nonintact bone and the intervening tissue at the nonunion site is often required [26].

Critical-size bone defect

A critical-size defect (CSD) is classified as the smallest bone defect that does not cure when surrounded by polymeric membranes (Fig. 1). To heal such defects in animals, it is necessary that membranes are applied in combination with autogenic bone graft and/or a proper bone substitute [19]. Ripamonti analysed the healing potential of calvarial defects in a series of adult baboons. They originate a distinct definition of CSD-dependent nonunion of the baboon calvaria [44, 45] and describe the defect as one that does not tend to heal spontaneously with

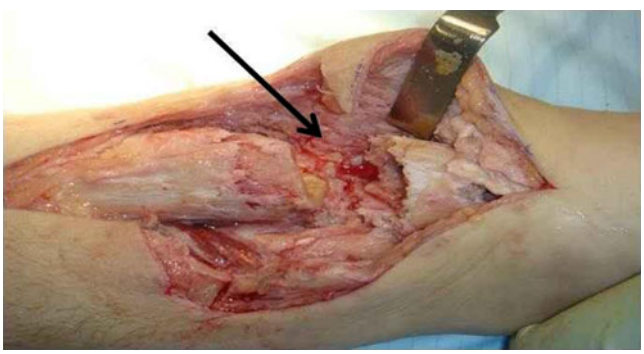

Fig. 1 A 5-cm tibial defect following debridement in an infected nonunion

bone and necessitates a bone graft or other substitute to heal.[44, 45, 48]

Another definition of this term was depicted as a segmental bone deficiency 2–2.5 times longer than the diameter of the injured bone. Not only the length of the bone defect but also other cofactors, such as anatomical site, associated soft tissue envelope, biomechanical-related hurdle in the injured bone, age, metabolic and systemic disorders and associated comorbidities, characterise a bone defect as critical [31]. Up to now, there is no consensus on a proper definition of CSD in humans. Several animal-based studies have been performed, but there are few clinical studies oriented towards managing CSD. The current definitions of CSD do not appropriately address the geometrical dimensions of the bones. According to our hypothesis, applying a distinct CSD index must contain information related to the length and diameter of the bone defect (Table 1).

Although each bone contains various degrees of vascularity and different soft tissue structures, which are not comparable for each bone, this concept might be effective, especially in cases of tibial fracture. Keating et al. [27] indicated that 0.4% of fractures were associated with bone loss for all fractures occurring between 1988 and 1999 in Edinburgh, Scotland. The majority of these fractures were classified as Gustilo grade IIIC. According to the authors' experience, the most common site of bone loss after fracture was the tibia. Whereas bone defects less than six cm long with adequate soft tissue coverage were bridged with conventional nails, plates and external fixation, defects over six cm were treated by shortening and fixation with later lengthening. If the soft tissue envelope was not injured, a combination of fixation and later bone transport was employed. In the presence of major muscle and nerve loss, amputation was suggested.

Generally, scores such as the Mangled Extremity Severity; Limb Salvage Index; Predictive Salvage Index; Nerve Injury, Ischemia, Soft Tissue Injury, Skeletal Injury, Shock, and Age of Patient (NISSSA) Score; and Hannover Fracture Scale-97 aid the surgeon in choosing between salvage and amputation. These scores tend to increase depending upon the severity of the injury. However, a multicentre prospective study did not confirm the clinical advantage of any of the lower-extremity injury severity scores. The authors concluded that the option to salvage should consider factors such as the general status of the patient and local injury to the limb [2]. In cases of segmental bone defects greater than four to five cm, with or without soft tissue defect, the literature advocates one of two methods: vascularised fibular grafting (VFG), and distraction osteogenesis or internal bone transport (IBT) with an external fixator (Ilizarov technique) [17, 30].

Table 1 Hypothesis of critical-size defect (CSD) index: all values are considered as appraised values

Bone segment	Diameter of the Bone (DB)	Length of the Bone Defect (LBD)	(LBD/DB) CSD Index
Humerus	2.0	2.0	1.0**
Ulna	1.0	2.0	2.0***
Tibia	3.0	2.0	0.6*
Femur	4.0	2.0	0.5*

Osteogenic potential of human MSCs

As is well known, human bone marrow, periosteum and fat tissue contain mesenchymal multipotent progenitor cells. Scarce amounts of MSCs with osteoblastic potency are also present in muscle, umbilical cord, placenta, dermis, cartilage and synovial fluid. The theory of osteoblast modulation is based on the idea that if situated in the proper milieu, a pluripotent cell will convey an osteoblast phenotype [8, 53]. As described by Chamberlain et al., the standard approach for differentiating MSCs into osteoblasts in vitro involves a several week incubation procedure that includes a mixed monolayer of MSCs with ascorbic acid, B-glycerophosphate and dexamethasone [4].

Bone remodelling occurs in two different places: within the trabecular and within cortical bone. As demonstrated by Hauge et al. in 2001 and Erikson et al. in 2007, remodelling occurs in exceedingly vascularised bone-remodelling compartments (BRC) [11, 20]. According to this model, cells destined to become osteoblasts enter the BRC via capillaries rather than from bone marrow. This scenario raises questions about the heritage of osteoblast precursor cells. A second model is based on the understanding that circulating osteoblastic cells add to the pool of osteoblastic cells entering the BRC. A final theory maintains that precursor cells within the capillary wall may also differentiate into osteoblastic progenitors [39]. As indicated by Eghbali et al., circulation of osteoblast-lineage cells correlates with markers of bone formation. As they are increased during puberty related growth, they are considered as a previously neglected circulatory component to the formation of bone, and they contribute to bone regeneration if harvested from peripheral blood, expanded in culture then implanted into the site of impaired bone healing [10]. Sambrook et al. indicated that high bone turnover is linked to increased cardiovascular mortality in elderly patients. That study indicated no correlation between age, sex, serum parathyroid hormone levels or hip-fracture status. Clearly, there is a potential link between neoangiogenesis and neo-osteogenesis [47].

To treat children with the progressive deformity osteogenesis imperfecta (OI), Horwitz et al. used a bone morrow transplant following moderately ablative chemotherapy. After three months, dense bone formed, total-body bone mineral content increased and reduced frequency of bone fractures with an increased growth velocity was observed [24]. That study implies that "mesenchymal progenitors" in transplanted marrow results in improved bone quality in patients with OI.

Indeed, MSC-based osteoblast differentiation undergoes several cycles and generates various intermediate products. The greater the differentiation status of the MSCs, the lower the proliferation ratio of the cells. Compared with the heritage of the MSCs, the local environment varies based on different stimuli, regulating cellular kinetics, gene expression and protein synthesis.

Review of clinical literature on nonunion and MSCs

A review of the literature revealed that, to date, there has been no study providing level I evidence regarding tissue engineering, bone marrow aspirates, demineralised bone matrices or gene therapy in humans [40].

Bone marrow aspirates

Connolly and Schindell presented the first outcomes for a case of infected nonunion of the tibia, followed by another publication on the application of marrow grafts for osteogenesis. The study included a 15-year follow-up of patients undergoing several different methods of marrow osteoprogenitor-cell application, including 100 patients with tibial nonunion. The study indicated that marrow grafts are beneficial for treating various skeletal healing-related problems. The method evaluated resulted in a better outcome compared to standard open iliac-crest grafting. The results indicated an option for stimulating osteogenesis in managing nonunion [6, 7]. Healey et al. presented good outcomes in eight patients treated by injection of autogenic bone marrow in situ. Patients with primary sarcomas were treated by extensive en bloc resections and reconstruction using internal fixation that developed delayed union or nonunion. These good clinical outcomes achieved under difficult clinical circumstances enhanced optimism regarding autogenic bone marrow grafting as a reliable procedure for treating nonunion [21]. Garg et al. performed percutaneous autologous bone marrow grafting in 20 cases of nonunion; in 17 cases, nonunion was fused within five months [14]. Goel et al. reported on clinical outcomes regarding the efficacy of percutaneous bone marrow grafting in patients with tibial nonunion and minimal deformity. They concluded that percutaneous bone marrow grafting is a "limited invasive technique" that is applicable under local anaesthesia and functions as a simple, safe, inexpensive and effective method in clinical cases of nonunion [18].

Concentrated bone marrow aspirate

Up to now, only one clinical study using concentrated bone marrow aspirate has been published. Hernigou et al. retrospectively evaluated 60 patients with noninfected atrophic nonunion of the tibia who had undergone percutaneous autologous bone marrow grafting [22]. Marrow was aspirated from both anterior iliac crests, concentrated in a cell separator and injected into nonunion sites. A positive correlation between the volume of mineralised callus at four months and the number and concentration of fibroblast colony-forming units in the graft was observed. In the seven patients who did not achieve fusion, both the concentration and the total number of stem cells injected were significantly lower than in patients with osseous union. The fracture gap between the ends of the fragments was less than five mm. Nonunion was assessed pre- and postoperatively by anteroposterior and lateral radiographs and computed tomography (CT). The volume of mineralised callus was calculated based on dimensions measured by CT. Once a callus appeared, weight bearing was recommended, and failure was considered when no healing occurred after six months. In an average of 12 weeks, a bony union was achieved in 53 of 60 patients. As is well known, osteocytes arise from colony-forming progenitor cells in the marrow. Hence, fibroblast colony-forming units (CFU) have been used as an indicator of stromal-cell activity. The average number of progenitor cells (± standard deviation) obtained from bone marrow was 612±134 progenitors/cm^3 and 2,579±1,121 progenitors/cm^3 after concentration. An average of 51×10^3 fibroblast CFU was inoculated into each nonunion site. The authors concluded that there was no relationship among factors such as age, sex, patient comorbidities and treatment outcome. Whereas types II and III open fractures required a longer time to heal (average 14 weeks), type I open and closed fractures needed only eight weeks on average. In addition, fracture location influenced healing time; i.e., distal fractures took longer to heal than proximal fractures. Patients with more comorbidities took a longer time to heal (average 14 weeks) than other patients (average ten weeks). The authors indicated that the concentrated buffy-coat layer included stem cells as well as other mononuclear cells that exhibited osteogenic or angiogenic properties, which affected the clinical outcome. One study limitation was the lack of a cohort with a placebo treatment. Accord-

ing to that study, a successful clinical outcome depends on the number and concentration of the stem cells being injected [22], (Table 2).

Other studies

Kim et al. performed a multicentre, randomised clinical study that included 64 patients with poor callus formation (lower than three points on the callus formation score) observed approximately six weeks after surgery. Patients were divided into two groups: one received an injection of autologous cultured osteoblasts and one had no treatment. Two months after surgery, autologous cultured osteoblasts were injected into the fracture area. The authors concluded that autologous bone transplantation represents an effective method of treating nonunion. However, taking into account the pain in the donor area induced by the surgical procedure, the limited volume of the bone graft and the additional surgery needed for an autologous transplant, an allogeneic osteoblast transplant presents a better option to promote bone union. The authors grafted autologous cultured osteoblasts to the nonunion area using fibrin, which enables osteoblasts to safely attach to the defect area and is promptly absorbed without inducing the reaction normally caused by foreign material [28]. Bajada et al. reported treating a nine year tibial nonunion resistant to six previous surgical interventions. They used autologous MSCs expanded to 5×106 cells after three weeks of tissue culture, followed by a combination of calcium sulphate in pellet form along with MSCs. Bony fusion was achieved two months after implantation. The authors showed that resorbable calcium sulphate (CaSO4) permits integration without affecting the vascular system [1]. Funk et al. reported treating an atrophic nonunion of the distal femur after correction osteotomy by applying periosteum-derived MSCs. The authors concluded that autologous periosteal bone precursor cells cultivated on a three-dimensional matrix composed of collagen can induce bone regeneration in a complicated case of nonunion, one for which several therapeutic attempts had previously failed. The benefit of this approach is based on the unlimited availability of cells that produce osteoinductive transplants after dissection of a small amount of periosteal tissue [13]. Iwakura et al. performed a study of seven patients with hypertrophic nonunion. Whereas intramedullary locking nails were used to treat five patients, plate-and-screw fixation was performed in one and external fixation in another. The presence of infection was excluded. All patients underwent their first nonunion operation. The time interval between the first operation and the operation for nonunion was nine to 14 months. Callus formation, thickening of the bones at the fracture ends and loosening of the fixation devices were observed in all cases. After dissecting the nonunion tissue, a sample of the tissue was processed using several tissue engineering procedures. To compare the population doubling time and the differentiation abilities of nonunion cells with those of fracture haematoma cells, fracture haematoma samples were attained from seven patients during osteosynthesis and cultured under the same conditions as the nonunion cells. The authors concluded that

Table 2 Clinical evidence for use of mesenchymal stem cells (MSC) in nonunion

Study and year bone marrow aspirate	Area of treatment	Level of evidence	No. of patients	Mode of administration and carrier	Healing times	Outcomes
Connolly and Schindell 1986 [7]	Tibial nonunion	III	100	100–150 ml marrow osteoprogenitor cells	6 to 10 months	Better outcome compared with standard open iliac crest grafting.
Healey et al. 1990 [21]	Patients with primary sarcomas that developed delayed unions or nonunions	III	8	50 ml marrow osteoprogenitor cell	4 to 36 weeks.	Good clinical outcomes achieved under difficult clinical circumstances
Garg et al. 1993 [14]	15 tibia, 3 humerus and 2 ulna nonunions	III	20 cases	Percutaneous autogenous bone marrow grafting (15–20 ml of bone marrow)	Five months	In 17/20 cases, nonunion healed
Goel et al. 2005 [18]	Tibial nonunion	III	20 cases	Percutaneous autogenous bone marrow grafting (15 ml of bone marrow	Fourteen weeks	In 15 cases, clinical and radiological bone union was achieved; four cases showed no sign of union
Concentratedbone marrow aspirate Hernigou et al. 2005 [22]	Tibial nonunion	III	60 cases	An average total of $51x10^3$ fibroblast colony-forming units was inoculated into each nonunion site.	Twelve weeks	Bony union was achieved in 53/60 patients.

Table 3 Clinical evidence for use of mesenchymal stem cells (MSC) in nonunion

Study and year other studies	Area of treatment	Level of evidence	No. of patients	Mode of administration and carrier	Healing times	Outcomes
Bajada et al. 2007 [1]	A 9-year tibial nonunion resistant to six previous surgical procedures	IV	1	Autologous bone marrow stromal cells expanded to 5×10^6 cells after 3 weeks' tissue culture, followed by a combination of calcium sulphate in pellet form with bone marrow stromal cells	Two months	Resorbable calcium sulphate permits integration without affecting the vascular system
Funk et al. 2007 [13]	Atrophic nonunion of the distal femur	IV	1	Once the defect has been stabilised via 90-grade plate osteosynthesis, it was filled with autologous cell matrix construct	Two years	Cultivated autologous peri osteal bone precursor cells on a three-dimensional matrix induces bone healing in a defect where various methods were unsuc-cessful to lead to union
Iwakura et al. 2008 [26]	Hypertrophic nonunion suffering from three femoral diaphysis, two tibial diaphysis , one humeral diaphysis and one ulnar diaphysis fractures	III	7	Nonunion tissue was incised into strips and cultured. Flow cytometry showed that the adherent cells were positive for MSC related markers CDl3, CD29, C044, C090, COlO5, C0166, and negative for the haematopoietic markers C014, CD34,C045, C0133, similar to control bone marrow stromal cells.		Hypertrophic nonunion tissue functions as a reservoir of mesenchymal cells that convert into cartilage and bone-forming cells
Kim et al. 2009 [28]	Long bone shafts of femur, tibia, radius, ulna and humerus	Multicenter, open, randomised, clinical study III	64 patients divided into autologous cultured osteoblast injection and no treatment groups.	A mixture with 0.4 ml (12×106 cells) and fibrin at the ratio of 1:1, placed in a syringe, and a 21-gauge spinal needle was placed into the syringe and injected into the fracture area	Difference in callus formation attained after 4 and 8 weeks,	Autologous cultured osteoblast transplantation presents an efficient method for increasing the rate of fracture healing

nonunion cells differentiate into osteogenic, chondrogenic and adipogenic cells in vitro. Hence, hypertrophic nonunion tissue functions as a reservoir of MSCs that can be converted into cartilage and bone-forming cells [26]. That study demonstrated that, in hypertrophic nonunion, fusion of the nonunion could occur without treating the nonunion site directly (Table 3), (Fig. 2).

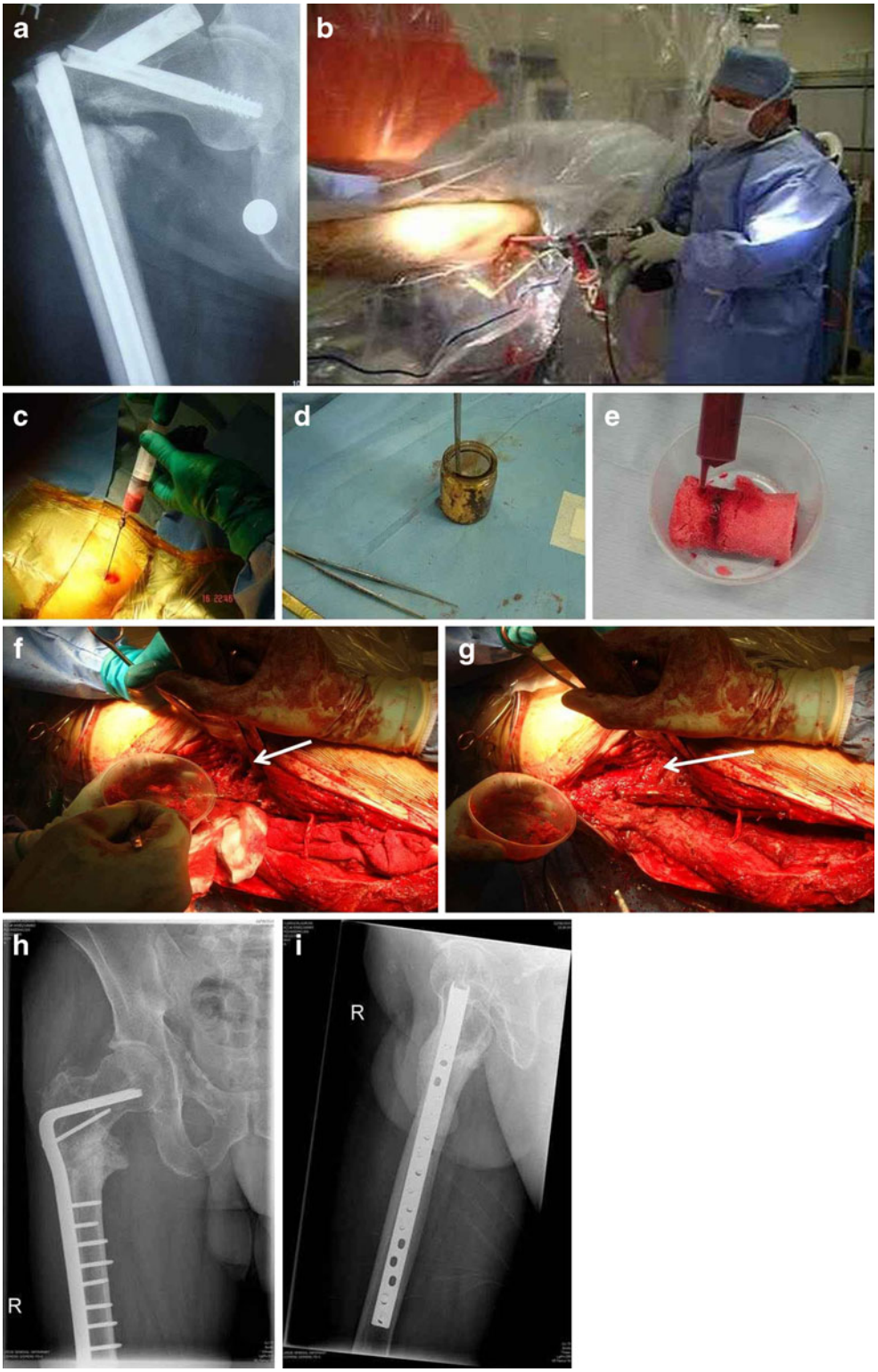

Fig. 2 **a** Anteroposterior radiograph of a right subtrochanteric femoral nonunion with a broken nail in situ 6 months following fixation. **b** Using the reamer/irrigator/aspirator (RIA) reamers, autologous graft was harvested from the left femoral canal. **c** Bone marrow aspirate was harvested from the left iliac crest. **d** Bone morphogenetic protein−7 (BMP-7) was prepared for implantation following dilution with 2 ml of sterile normal saline. **e** RIA graft was mixed with the concentrated bone marrow aspirate. **f** Nonunion was stabilised with a blade. The RIA graft mixed with the bone marrow aspirate and the BMP-7 was implanted at the site of the nonunion (*white arrow*). **g** Nonunion site (*white arrow*) was filled with the graft material. **h**, **i** Anteroposterior and lateral radiographs of the right femur 4 months after the operation illustrating healing of the previous nonunion

Review of clinical literature on large bone healing defect and MSCs

In 2001, Vacanti et al. reported a case of a 36-year-old patient with dorsal skin, nail, nail bed, extensor tendon and distal phalanx of his left thumb torn off in a machine accident. After thorough debridement, the wound was covered by a pedicle of abdominal skin. For this process, 8 cm^2 of periosteum was harvested from the distal part of the left radius and cultured ex vivo for nine weeks. Three months following the injury, the skin graft on the dorsum of the thumb was incised longitudinally. A pocket was built beneath the flap and filled with a scaffold complex composed of porous hydroxyapatite that was injected with a cell suspension containing periosteal cells. Afterwards, the thumb was splinted for eight weeks. Ten days after receiving the implant, the patient was able to use his hand. The authors indicated that effective tissue engineering involves implanting living cells with synthetic scaffolding. In that case, the coral scaffold was seeded with periosteal cells. To some experts in reconstructive hand surgery, this technique was considered quite time consuming for a simple clinical case. However, from our perspective, this technique has had a pronounced effect on combining some therapeutic concepts from the fields of hand and reconstructive surgery with tissue engineering [52]. Quarto et al. and Marcacci et al. applied cultured, expanded stem cells to treating four patients. Osteoprogenitor cells were obtained from bone marrow and cultured ex vivo. These cells were placed on macroporous hydroxyapatite scaffolds, the size and shape of which were adjusted according to the particular bone defect in each patient, and the cultured cells were implanted at the lesion sites. Initially, the defect was repaired by applying an external fixation that was later removed. The authors concluded that the application of culture-expanded osteoprogenitor cells grown on porous bioceramic scaffolds results in substantial improvement in the repair of large defects in long bones [34, 49]. Evidence indicates that direct application of MSCs is promising. This potential was also demonstrated in a study related to bone-healing defects in osteogenesis imperfecta [18]. However, all of these studies lacked control groups.

Kitho et al. retrospectively reported on the outcomes of a clinical study that included two groups of patients with an average age of 15.0±3.21 years: 51 patients undergoing lower-limb lengthening were treated with bone marrow cells (BMC) and platelet-rich plasma (PRP) between the femur and tibia and compared with a control group of 60 patients who received no cell therapy. The treatment procedure was based on BMC expansion and differentiation into osteoblasts by culturing them in a differentiation medium. PRP was prepared from venous blood by centrifugation. For each patient, this treatment regimen

Table 4 Clinical evidence for using mesenchymal stem cells (MSC) in large bone healing defect

Study and year	Area of treatment	Level of evidence	No. of patients	Mode of administration and carrier	Healing times	Outcomes
Quarto et al. 2001 [49]	Large bone diaphysis defect (3.0–28.3 cm^3)	III	4	Cells obtained from the patient's marrow were expanded in culture and seeded onto hydroxyapatite ceramic scaffold followed by a stabilisation via external fixation	5–7 months	Application of culture-expanded osteoprogenitor cells in combination with bioceramic succeeded
Marcacci et al. 2007 [34]	1 Tibia, 1 Humerus, 2 separate Ulnar fractures					
Kitoh et al. 2009 [29]	23 femora, 28 tibiae	III	51 compared with 60 controls without MSC and PRP	Application of ex vivo expanded MSC combined with PRP	Average healings index of bones treated with BMC and PRP (30.0± 6.72 d/cm) was significantly lower than that of bones without cell therapy (51.4±26.5 d/cm) both in the femur ($P<0.0001$) and in the tibia ($P=0.0059$)	Locally varying bone-forming development by cell transplantation is dependent on regional blood supply and soft tissue covering.

PRP platelet-rich plasma, *BMC* bone marrow cells

cost more than US $2,000, including personnel expenses, culture instruments and chemicals and contamination tests. Indeed, a detailed cost–benefit analysis of this treatment is recommended. As BMC and PRP are both autologous, this procedure appears to be a safer and less toxic and immunoreactive approach. Whereas faster femur lengthening was observed compared with tibial lengthening, the healing index of the BMC and PRP group was significantly lower than that of the control group. In contrast to the findings of Hernigou et al. [22], Kitho et al. found no correlation between the healing index and the amount of transplanted cells or PRP concentration. The authors pointed out that beneficial outcomes related to bone regeneration by therapy based on BMC and PRP can be expected only when cells are transplanted into an area with adequate blood supply and copious soft tissue. In addition, locally varying bone-forming processes need to be taken into consideration [29] (Table 4).

Diamond concept of bone-fracture healing

To regenerate hard and soft tissue deficiency, a triangular-shaped model of osteogenic cell groups, osteoinductive stimulants and osteoconductive matrix is an adequate technique. Mechanical stability was added to the diamond model for bone-fracture healing [16]. Hence, the diamond concept can be applied to treating nonunions and large bone defects. Masquelet et al. performed a study in a series of 35 adult patients and employed a novel two-stage technique comprising insertion of a cement spacer, induction of a membrane, and reconstruction of the defect with cancellous bone autograft [35]. Indeed, applying biological pseudomembranes has demonstrated the effectiveness of the diamond concept in the clinical scenario. Reconstruction of bone defects up to 25 cm long enabled normal walking in an average of 8.5 months after weight-bearing diaphyseal segment reconstruction [36]. The induced pseudomembrane not only functions as protection but also as a vascularising agent for the bone graft. It has been demonstrated that the induced pseudomembrane is copiously vascularised by numerous capillaries in all layers. Whereas high concentrations of vascular endothelial growth factor (VEGF) and transforming growth factor B (TGF-B) were generated as early as the second week, the concentration of bone morphogenetic protein-2 (BMP-2) inside the membrane was increased to the highest level at the fourth week [42]. Early stages of the pseudomembrane formation accompanied by secretion of growth factors indicate the essence of the pseudomembrane role as an in situ growth-factor delivery system that strengthens bone-graft healing. Nevertheless, it is a two-phase procedure with its associated risks. Moreover, options are few for large-defect autograft. This limitation has been overcome with the application of the reamer/irrigator/aspirator (RIA) and the option of harvesting autologous graft in large amounts from the inner cavity of the femur. Thus, performing more clinical studies that use the diamond concept to further improve reconstruction of large bone defect is essential [15].

Conclusion

Blood vessels apparently function not only as nutrient suppliers but also as an important source of perivascular MSCs that consequently differentiate into osteoblasts. Indeed, vascularisation contributes tremendously to the formation of new bone. Symbiosis between angiogenesis and osteogenesis ensures appropriate bone repair [3, 33]. There is increasing indication that cells of nonmesenchymal origin (e.g. endothelial progenitor cells) augment bone and other tissue regeneration. Maes et al. applied tamoxifen-inducible transgenic mice bred with Rosa26R-LacZ reporter mice to study the fates of stage-selective subsets of osteoblast lineage cells during embryonic bone development and adult fracture repair. They reported that although cartilaginous callus forms following a fracture in the absence of a blood vessel, the substitution of cartilage by bone only can occur following the invasion of blood vessels into the callus [51]. In summary, the essence of bone regeneration consists of a combination of biological and biomechanical therapeutic approaches. Whereas biomechanical treatment options for nonunion and bone healing defects are widely available, biological resources appear to be limited. Evidence indicates that a combination of factors stimulating neo-osteogenesis and neovascularisation will restore hard and soft tissue deficits. It appears that the optimal approach involves a combination of a biomaterial scaffold, cell biology techniques, a growth factor, an optimum mechanical environment (diamond concept) and adequate surgical intervention [12]. To clinically demonstrate the effectiveness of this proposed approach after bone injury, additional clinical studies are required, which should examine the roles of the biomaterial scaffold and various concentrations of orthobiologics and their impact on skeletal cell regeneration.

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