Clinical Orthobiologic Approach to Failure or Delay in Bone Healing

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Content

Great part of the fractures heal spontaneously in the expected timing, if correctly treated, but approximately 5–10% don't, with an incidence of 19 per 100,000. [1] Delayed unions and non-unions of long bone fractures, the latter defined by the Food and Drug Administration as fractures for which a minimum of 9 months has elapsed since the injury and for which there have been no signs of healing for 3 months, represent an important therapeutic challenge for the orthopaedic surgeons, but also an important social economic burden due to the morbidity, the costs and the disability to work that these conditions cause. Already in 1995, Einhorn and co-workers reported that, in the United States, of about 5.6 millions fractures treated, up to 10% do not heal completely [2] and this requires several complex and long-lasting type of treatments. Looking to what this means in terms of costs, in the UK, Dahabreh, Dimitriou and Giannoudis, in 2011 [3], reported that the treatment of one single case of non-union requires 13.844,68 pounds that is well related to what reported some years before in 2005 [4] for the costs sustained in Canada to treat a tibial shaft fracture, equal to 18.712 Canadian dollars. This pattern was ulteriorly emphasised in the epidemiological study conducted on 5,169,140 Scottish population, published in 2013, where the cost to the National Health Service of United Kingdom of treating a non-union has been reported to range between £7000 and £79,000 [5]. The authors reported that 4895 non-unions

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were treated as inpatients in Scotland between 2005 and 2010, averaging 979 per year, with an overall incidence of 18.94 per 100,000 population per year, with the gender distribution of 57% incidence in male and 43% in female and an overall age peak incidence in the fourth decade of life. Extrapolating from Scottish figures of 1000 cases of non-union per annum, the incidence of non-union in the United Kingdom is around 11,700 cases per annum. This would suggest that non-union costs the health services in the United Kingdom alone several hundreds of millions of pounds per year [6].

The cause of non-unions is still not definitely clear: the origin of this type of pathology is retained to be multifactorial. In fact, several causes have been proposed in the ethiology and can be related to metabolic modifications or comorbidities of the patient, such as diabetes, obesity and smoking [7], a trauma, a local unfriendly environment and an altered biological pathway hindering the normal healing process. Once these types of injuries were retained to be a major concern in developed countries, but more recently traffic trauma fractures have become an important social impact also in less developed countries, where they even cause more frequent major disabilities, such as joint stiffness, malunion and non-union [8].

Classically, delayed unions and non-unions have been treated by several methods of stimulating bone repair, such as bone grafting, mostly autologous but also allogenic, or synthetic bone substitutes. Autologous bone grafting has always been retained as the gold standard in non-union treatment and several types of grafts have been proposed and used, such as cancellous, corticocancellous, segmental and vascularised, mostly, fibula bone grafts. Every type of different graft has presented its own specific indications and limitations. Autologous cancellous bone is widely regarded as an ideal construct for graft procedures, supplying osteoinductive growth factors, such as bone morphogenetic proteins, osteogenic cells and an osteoinductive scaffold, and has a successful rate of 50–80% [9]. However, its use is limited by the fact that its harvesting is an invasive procedure associated to donor site morbidity and has limited available procurable quantities. Allografts (fresh-frozen, dried or lyophilised) are available in many forms, such as demineralised bone matrix, cancellous and cortical, corticocancellous, osteochondral and whole-bone segments [10], have no limits in graft quantities but present the risk of transmission of infectious diseases, potential minor immunogenic rejection and post-operative infections and refractures. Although synthetic grafting materials eliminate these risks, these materials, such as bioceramics, hydroxyapatite and tricalcium phosphate, do not transfer osteoinductive or osteogenic elements to the host site and their degradation can be influenced by the anatomical location and several clinical conditions. To offer the advantages of autograft and allograft, a composite graft may be considered. Such a graft can combine a synthetic scaffold with biologic elements to stimulate cell infiltration and new bone formation [11]. A particular solution has proposed, in the treatment of acetabular revisions with bone allografting, the combination of an allograft with mesenchymal stem cells (MSCs) contained in bone marrow concentrate [12]. Sixty patients have been divided into two groups: 30 patients received the allograft + an average of 195,000 cells (range 86,000–245,000 cells) while the control group (30 patients) received only the allograft. The radiographic analysis, at a minimum of 12 years follow-up, showed better graft union and less allograft resorption in the group treated by allograft plus MSCs.

As stated earlier, fracture healing is a multidimensional process consisting of different, well-established and overlapping phases: an initial inflammatory response, soft tissue callus formation, hard callus formation, initial bony union and bone remodelling. These processes mostly occur contemporarily with alternating anabolic bone formation and catabolic remodelling processes. The latter catabolic phase consists in the removal of the soft, cartilaginous callus, initially formed, and its remodelling in the hard bony callus.

Studying deeply at the cellular level this complex repairing process, we find several cellular types involved: inflammatory cells, vascular cells, osteochondral progenitors including mesenchymal stem cells and osteoclasts [13].

Mesenchymal stem cells are defined as non-haematopoietic stromal cells that contain multilineage differentiation ability and are capable of stimulating the growth of bone, cartilage, adipose tissue, tendons and muscles [14, 15]. These pluripotent cells are found in multiple human adult tissues including bone marrow, synovial tissues, adipose tissues, umbilical cord and placenta.

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy [16], in order to propose a common definition, has proposed minimal criteria to define human MSCs:

- MSC must be plastic-adherent when maintained in standard culture conditions
- MSC must express CD105, CD73 and CD90
- MSC lacks expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules
- MSC must differentiate to osteoblasts, adipocytes and chondroblasts in vitro
- MSC lacks of expression of haematopoietic antigen

Interestingly, in 2009, Iwakura et al. [17], studying the histology of hypertrophic non-unions, investigated whether the cells derived from non-union tissue had the capacity for multilineage mesenchymal differentiation. Flow cytometry revealed that the adherent cells were consistently positive for mesenchymal stem cell related markers CD13, CD29, CD44, CD90, CD105, CD166, and negative for the haematopoietic markers CD14, CD34, CD45 and CD133, similar to control bone marrow stromal cells. In the presence of lineage-specific induction factors, the adherent cells differentiated, in vitro, into osteogenic, chondrogenic and adipogenic cells. The authors were, therefore, able to conclude that their results demonstrated that hypertrophic non-union tissue contains multilineage mesenchymal progenitor cells and that fracture site serves as a reservoir of mesenchymal cells that are capable of transforming into cartilage and bone forming cells.

The bone healing process of fractures and small bone defects has been ulteriorly defined by Giannoudis and co-authors [18] that, in 2007, introduced the "diamond concept" of fracture healing. This process involves a cascade of events and well-orchestrated interactions between several actors. First of all, mesenchymal stem cells are recruited at the fracture injury site or transferred to it with the blood circulation. The fracture haematoma has been proven to be a source of signalling molecules (interleukins/IL-1, IL-6, tumour necrosis factor-a/TNF-a, fibroblast growth factor/FGF, insulin-like growth factor/IGF, platelet-derived growth factor/PDGF, vascular endothelial growth factor/VEGF and the transforming growth factor β/TGFβ superfamily members) that may be the inducer of the cascade of cellular events that initiate healing [19]. All these growth factors are secreted by many of the present cells, including mesenchymal stem cells and osteoblasts [20]. The third element of fracture healing is the extracellular matrix that provides the natural scaffold for all the cellular interactions.

Various osteoconductive matrices and different biomaterials such as collagen, demineralised bone matrix, allografts, hydroxyapatite, polylactic or polyglycolic acid, bioactive glasses and calcium-based ceramics have been used as bone void fillers. The fourth element is the mechanical stability of the fracture environment with a good blood supply that is essential in order to promote callus formation and fracture healing. All the fracture fixation devices are applied in the goal of promoting biological fracture stability and preserving the essential soft tissue envelope and vascularisation of the fracture gap. This pursuit of mechanical stability has become always more emphasised, varying specifically and also depending from the particular graft or scaffold being used, that may require different specific environments, based on the characteristics both of the host bone than of the implanted material.

These complex cascade of events require a precise orchestration of the distinct phases that overlap one another till fracture union and bone remodelling are completed. However, physiological callus formation can be derailed by a variety of factors, including menopause-associated hormonal changes, age-related factors, changes in physical activity, drugs and secondary diseases, which lead to the development of various bone disorders in both women and men. Physiological callus formation can, also, be modified by other factors, such as the presence of inflammation, the size of the gap between the fracture bone ends, loading of the fracture and the presence of osteoprogenitor cells [21]. If all the processes progress smoothly through the "bone healing cascade", the fracture heals completely and normally and the bone segment remodels. During the initial phase after a fracture, many cytokines are released and attract different type of cells, from the endothelial cells and fibroblast to mesenchymal stem cells that promote chondrocyte and osteoblast proliferation and extracellular matrix production [22].

The progressive understanding and knowledge of the cellular pattern activated at the fracture level has promoted the introduction of an alternative solution to the use of autografts, allografts or scaffolds: in the recent years the recourse to regenerative medicine and the use of mesenchymal stem cells withdrawn from bone marrow or adipose tissue has become an attractive and useful therapeutic solution proposed also in the treatment of delayed fracture healing, nonunions and bone defects. As described before, MSCs have an important part in fracture repair. Osteoprogenitor cells can be implanted in large numbers in the traumatised area, alone or in association with a scaffold. Autologous bone marrow contains growth factors and osteoprogenitors cells, since mesenchymal stem cells are present in the mononuclear cellular fraction of the bone marrow and they can be readily obtained by culturing the anterior or posterior iliac crest aspirates. Over the years, several mesenchymal stem cell based therapies have been developed both with (increasing the number of cells to millions of mesenchymal stem cells) and without cell culturing and with or without a scaffold, and concentrated or non-concentrated mononuclear cells can be mixed in the operating room with natural or synthetic osteoconductive scaffolds before the implantation.

Bone marrow has represented the first and most diffuse source of cells used to favour delayed fractures and non-unions healing.

Already in 2007, Meijer et al. [23] stated that more than 300 papers about bone tissue engineering in rodents had been published indicating the feasibility of the technology and shown successful results. For example, Kadiyala et al. [24] implanted culture-expanded autologous bone marrow mesenchymal stem cells loaded onto porous ceramic cylinders into 8 mm segmental defects in rat femora. This resulted in significantly more bone fill and new bone formation at 8 weeks in the mesenchymal stem cell loaded implants compared to control cylinders loaded with bone marrow or cell-free cylinders. A study on adipose-derived stem cells cultured in osteogenic media on polylactide-*co*-glycolic acid showed successful treatment of 8 mm calvarial defects in rats [25]. Diversely, just a limited number of studies had been reported, at that time, on the use of mesenchymal stem cells for the treatment of osseous defects in larger animals, but all of them claimed a successful bone formation in long bones, cranial and mandibular defects of sheep [26–28], femoral defects in dogs [29], and iliac wings of goats [30]. In particular, in the paper by Bruder et al. [29], it was demonstrated that the implantation of bone marrow mesenchymal cells supported osteogenesis over an empty scaffold, including the formation of reparative callus, absent in defects treated just with the scaffold without any cell.

In 2013, a systematic review [31] analysed a total of 503 articles and found 23 articles relevant on preclinical and a very limited number of clinical studies on the use of scaffolds for bone repair in skeletal defects: the authors concluded that the adjunct of mesenchymal stem cells to scaffolds enhances osteogenesis when treating bone defects.

More recently, a large systematic review of 20 studies [32] has analysed the treatment outcomes reported in the preclinical studies on 406 large

animals (pig, dog, sheep or rabbit) in which bone defects were treated with stem cells therapies, of various origin (bone marrow mesenchymal stem cells (BMSCs), umbilical cord blood mesenchymal stem cells (UCB-MSCs), deciduous teeth stem cells, adipose stem cells (ASCs)), analysing also the results on the basis of number of cells injected (<107 or ≥107), method of cell delivery (cell seeded on scaffold, in situ injection, or intravenous administration) and follow-up period after stem cell therapy (≤12 weeks, 12–24 weeks or >24 weeks). The evaluated results have been considered "conflicting, with some studies reporting bone regeneration when stem cells are used alone [33, 34] or in combination with scaffolds [35, 36], while other studies failed to find significant differences" [37, 38]…. The authors then concluded: "Although these preclinical studies remain controversial, the results offer important clues to unanswered clinical issues which are critical to stem cell repair of the bone including safety, feasibility, efficacy, choice of cell type, cell number, method of delivery and follow-up".

In fact, they reported that stem cell therapy promoted new bone formation by 17.79% accompanied with BMD increase of 276.94 mg cm^{-2}, cell injection dose and the route of cell delivery were important predictors of new bone formation in the bone defect model and that in animal and stem cell types no differences were found. Transplantation of a higher number of cells (≥107) appeared to have a stronger impact on new bone formation. This could be the expression of the paracrine stem cell capacity [39] that stimulates the endogenous regenerative capacity through the activation of growth factors and cytokines. Clinically, the addition of bone marrow derived stem cells didn't show any benefit when compared with other stem cells on new bone formation. Compared with BMSCs, ASCs and UCB-MSCs have several advantages as new cell sources including ease of isolation, relative abundance, rapidity of expansion and multipotency [40, 41].

The first reported studies on the treatment with mesenchymal stem cells in humans reported, in 2001 and 2003, the treatment of three patients with segmental defects of long bones (4 cm bone segment loss in the right tibia, 4 cm in the right ulna and 7 cm in the right humerus), using ex vivo expanded human MSCs, loaded on a three-dimensional scaffold of the shape and size of the missing bone fragment [42, 43]. External fixation was provided for stability and removed after 6.5, 6 and 13 months, respectively, in patient number one, two and three. All three patients presented a repair of the fracture site: the implants showed good integration of the newly formed bone and abundant callus formation on follow-up radiographs and CT scans.

In 2004, the following published clinical study described the augmentation procedure of the posterior maxilla in 27 patients, using bone matrix derived from mandibular periosteum cells on a polymer fleece. In 18 patients, an excellent clinical, radiologic, and histologic (mineralised trabecular bone with remnants of the biomaterial) result could be proved 3 months after augmentation. In eight patients, an unsuccessful outcome was observed with replacement resorption with connective tissue [44].

In 2008, Shayesteh et al. [45] treated six cases of sinus augmentation using human bone marrow aspirate expanded mesenchymal stem cells loaded into a biphasic beta-tricalcium phosphate/hydroxyapatite scaffold. Of 30 implants, 28 (93%) were considered clinically successful. Histologic evaluation of the biopsies revealed numerous areas of osteoid and bone formation on the scaffold, in absence of any complications. All implants were considered clinically and radiographically osteointegrated after 4 months. The findings suggested that the addition of MSCs to bone derivative/substitute materials may enhance bone formation.

Then, in 2010, Grayson and co-workers [46] tested the possibility to engineer anatomically correct pieces of fully viable and functional human grafts and succeeded in obtaining the filling of temporomandibular joint defects using bone marrow mesenchymal stem cells and a "biomimetic" scaffold-bioreactor system.

In following years, tissue engineering has continued to be studied and considered as a new possible useful addiction to standard care of non-unions [47, 48].

Bajada et al. [49] treated a 9-year-old tibial non-union, that had undergone six previous operative attempts to treat it, using bone marrow stromal cells expanded to 5 × 10(6) cells after 3 weeks tissue culture and a calcium sulphate scaffold. The non-union was clinically and radiologically healed 2 months after implantation.

Giannotti et al. [50] examined the long-term efficacy and safety of ex vivo expanded bone marrow mesenchymal stem cells embedded in autologous fibrin clots and implanted with bone grafts for the healing of atrophic pseudarthrosis of the upper limb in eight patients. All patients recovered limb function, with no evidence of tissue overgrowth or tumour formation. Authors traced an important conclusion: "The respect of tissue geometry, the stability of healing and the absence of neoplastic transformation at such long-term follow-ups underline the feasibility and safety of this procedure within the frame of a regenerative medicine approach".

Fernandez-Bances et al. [51] successfully treated seven patients with long bone non-unions with autologous bone marrow mononuclear cells withdrawn from iliac crest combined with frozen allogenic cancellous bone graft. All patients showed complete bone consolidation at a mean of 5.3 months associated to limb pain disappeared. At a mean follow-up of 35.8 ± 4.6 months after transplantation (range, 24–51 months), there was no recurrence of pseudoarthrosis or pain.

Applying the concept of growth factor stimulation, Grgurevic et al. [52] showed that exposure of bone marrow stromal cells to growth factor such as BMP1-3 (recently discovered to be significantly increased in patients with acute bone fracture) increased the expression of collagen type I and osteocalcin in MC3T3-E(1) osteoblast like cells, and enhanced the formation of mineralised bone nodules in rat long bone non-unions.

Gomez-Barrena et al. [1], in reporting on the ongoing clinical trials on bone fracture and nounion treated with mesenchymal stem cells, were able to identify 13 trials as completed or recruiting patients and divided these in four groups: the first on the percutaneous injection of bone marrow aspirate concentrate (BMAC): none of the studies was published; the second group included patients treated by BMAC associated to bone substitutes or demineralised bone matrix: in this group one paper [53] was published and showed a shorter time to obtain bone union with cells respect the controls. In the third group, three trials studied the percutaneous injection of expanded mesenchymal stem cells but no paper had still been published. In the fourth group, the association of expanded mesenchymal stem cells and bone matrix or substitute was studied, but the only completed study was not yet been published.

Percutaneous bone marrow grafting is a minimally invasive treatment. It avoids the complications associated with the open graft harvest procedure. Connolly and colleagues [54–56] have been the first to demonstrate the efficacy of percutaneous bone marrow injection in the treatment of non-united fracture of the tibia. Healey et al. [57] presented good outcomes in eight patients with non-unions of failed fixation of primary sarcomas treated by injection of autogenic bone marrow in situ. Garg et al. [58] performed percutaneous autologous bone marrow grafting in 20 cases of non-union; in 17 cases, non-union was fused within 5 months. In a cohort of 20 tibial non-unions, 90% healed in average of 6 months after the injection. In a retrospective study involving 60 atrophic tibial non-unions, Hernigou et al. [59] demonstrated complete healing in 88.3% that were treated with a single injection of bone marrow aspirate. Goel et al. [60] reporting on the efficacy of percutaneous bone marrow grafting in patients with tibial non-union and minimal deformity stated 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 non-union.

However, this technique, if used alone, may not be sufficient to induce healing of complex fractures with large bone gaps [61].

Very recently, Ismail et al. [62] published a comparative study on the treatment of ten patients with neglected atrophic non-union of a long bone fracture divided in two groups of five patients each: the first group treated by the combination of 15 million autologous bone marrow mesenchymal cells harvested as outpatients from the posterior iliac crest and cultivated for 4 weeks till reaching the desired number of cells, HA granules and internal fixation; the second group was treated by iliac crest autograft, HA granules and internal fixation. The first group showed faster initial radiographic and functional improvements (VAS, LEFS and DASH scores). The first group reached radiographic consolidation at 8 months, 3 months earlier than the second group. The functional scores between the two groups converged after the seventh month. The authors retained that percutaneous injection of the stem cells may risk losing a substantial amount of cells by apoptosis due to lack of cellular attachment. They also stated that, even if a good biological environment provided by preserved soft tissue is beneficial for the bone healing process, in most long-standing cases of non-union decortication of the fracture site, provided by the surgical procedures, is essential. This procedure has the goal to provide an active biological chamber [63] to support the physiological healing process, confirming the diamond concept of fracture healing [18].

However, the use of bone marrow or bone marrow concentrate mesenchymal stem cells may be limited due, principally, to the morbidity associated to the harvest and to the relative small number of cells that can be withdrawn by this technique. In contrast, adipose tissue stem cells can be easily harvested by liposuction with low donor morbidity and the chance to obtain a large number of cells with less limitations of donor's age. In fact, the frequency of stem cells within the adipose tissue is reported to be 500 times greater than that of bone marrow [64]. Thanks to these properties, adipose-derived stem cells (ASCs) currently are becoming more and more used in a variety of clinical applications. Subcutaneous adipose tissue is rich in mature adipocytes (67.6%), but it is also composed of blood vessels, leukocytes, fibroblasts, macrophages and pre-adipocytes, identified as Stromal Vascular Fraction (SVF) [65–67]. Each adipocyte is completely surrounded by a capillary system and this gives the explanation for the fact that the number of mesenchymal stem cells in adipose tissue is so higher in respect to the bone marrow's one [68, 69]. In fact, it is now accepted that the microvascular perycites represent the precursors of the mesenchymal stem cells [70–73]. The identification of the stroma and the possibility to use this stromal vascular fraction, with its high prevalence of stem/stromal cells, for therapeutic uses, have made the adipose tissue a suitable source for clinical applications.

The study of adipose stem cells for bone regeneration has been associated to the implantation of this type of cells on several scaffolds and has been studied in rat and nude mouse models [74–76]. However, relatively few reports are available concerning the use of adipose stem cells for human bone tissue regeneration [77].

The first paper supporting the clinical application of a human adipose stem cells on a scaffold to promote fracture healing was reported by Lendeckel et al. in 2004 [78]. This is a report of a 7-year-old girl suffering from wide calvarial defects after severe head injury with multifragment calvarial fractures. Due to the limited amount of autologous cancellous bone available from the dorsal iliac crest, autologous adipose-derived stem cells withdrawn from the gluteal region were processed simultaneously and applied to the calvarial defects in a single operative procedure together with autologous fibrin glue applied through a spray adapter to keep the cells in place. Follow-up CT scans showed new bone formation and near complete calvarial continuity 3 months after the reconstruction.

In 2009, Mesimäki et al. [79] described a novel method to reconstruct a major maxillary defect in an adult patient with a microvascular flap using autologous human mesenchymal stem cells, combined with recombinant human BMP-2 and β-tricalcium phosphate granules. After 8 months of follow-up, the flap had developed mature bone structures and vasculature

and was transplanted into the defect area. Several other authors have reported good results in the treatment of craniofacial osseous defects with adipose stem cells and resorbable scaffolds [80–83].

In 2016, Tawonsawatruk et al. [84] compared human bone marrow derived mesenchymal stem cells and human adipose-derived pericytes delivered percutaneously to the fracture gap to prevent the formation of atrophic non-union in a three group rat model (bone marrow cells group, adipose perycites group and no cell control group). At 8 weeks, 80% of animals in the cell treatment groups showed evidence of bone healing compared to only 14% of those in the control group. Radiographic parameters showed significant improvement over the 8-week period in the cell treatment groups, and histology confirmed bone bridges at the fracture gap in the both treatment groups. The quality of bone produced and its biomechanical properties were significantly enhanced in both cell treatment groups. These results brought to the conclusion that MSCs and pericytes have significant bone regeneration potential in an atrophic non-union model: "These cells may have a role in the prevention of atrophic non-union and can enable a paradigm shift in the treatment of fractures at high risk of failing to heal and developing non-union". This study demonstrates also that pericytes can be harvested from adipose tissue in sufficient numbers for immediate autologous use without the requirement for culture. It is therefore possible to use pericytes for one-step cell-based therapies, within a single intraoperative approach, because sufficient numbers of cells can be sorted immediately from adipose tissue and implanted back to the fracture site.

As stated earlier to overcome the limits of autologous bone grafting, scientists have looked at the possibility of autologous cell-based engineered bone grafts. These procedures are challenged by the complexity, impracticality and high costs of the manufacturing process [85]. This is predominantly due to the need for two surgical procedures (respectively for cell harvest and graft implantation) and extensive ex vivo cell manipulation and culture under good manufacturing production regulations/facilities. To simplify the engineering of autologous osteogenic grafts, this procedure must become a one-step procedure. This has been studied using bone marrow mesenchymal stromal cells, either immunoselected for CD105 [86] or in combination with a gene therapy approach [79].

In 2007, Helder and collegues proposed the use, in this one-step regenerative surgeries, the use of stromal vascular fraction, because of the higher number of stem cells available and because SVF includes endothelial cells that could help graft vascularisation [87]. Muller et al. [88] have confirmed that the stromal vascular fraction of human adipose tissue from lipoaspirate can be used intraoperatively to generate autologous cell-based therapies for bone repair, developing, in 3 h, osteogenic and vasculogenic grafts using human SVF cells in a setting compatible with an intraoperative clinical implementation.

In conclusion, in the last years, tissue engineering has seen a multiplication of its applications in Orthopaedics and Traumatology with the goal of improving repair and regeneration of bone, cartilage, tendon and muscle lesions and this has been associated with an increasing number of in vitro and in vivo reports in the literature. The capacity of identifying, isolating and using mesenchymal stem cells withdrawn from various tissues, designing matrices and scaffolds able to favour the growth of these cells and deliver them in situ and the adjunt of growth factors can provide a very useful tool to accelerate and complete the healing processes. In particular, bone healing requires viable biologic environment, mechanical stability, osteogenic cells and growth factors than can initiate and stimulate the recovery process. The regenerative strategies that use mesenchymal stem cells are showing promising results in improving the biology of the site of acute, delayed and non-united fractures. These therapies in the future will certainly continue to improve the bone formation cascade, enabling orthopaedic surgeons to reduce the timing, the morbidity and the social costs of these pathologies, ameliorating the quality of life of an increasing ageing work population.

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