Medical Hypotheses 111 (2018) 4–7

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

A novel bimodal approach for treating atrophic bone non-unions with extracorporeal shockwaves and autologous mesenchymal stem cell transplant

Valerio Sansone[a,b,*], Manuel Brañes[c], Pietro Romeo[b]

[a] Department of Orthopaedics, Università degli Studi di Milano, Via Festa del Perdono, 7, 20122 Milan, Italy
[b] Galeazzi Orthopaedic Institute, Via Riccardo Galeazzi, 4, 20161 Milan, Italy
[c] Universidad de Chile, Facultad de Ciencias, Las Palmeras 3425, Ñuñoa, Región Metropolitana, Chile

ARTICLE INFO

Keywords:
Extracorporeal shock wave therapy
Atrophic
Non-unions
Mesenchymal stem cells
Bone marrow stromal cells
Autologous transplant

ABSTRACT

We propose a novel approach for the treatment of atrophic bone non-unions via parallel applications of extracorporeal shock wave therapy (ESWT) and an autologous mesenchymal stem cell transplant. The hypothesis resides on the potentiality of shock waves (SWs) to act as a tool for manipulating the patient's mesenchymal stem cells (MSCs). In addition to the conventional physical stimulus achieved by delivering SWs at the site of non-union to stimulate the well-known trophic effects on bone tissue, a series of concomitant ESWT would be administered in tandem at a bone marrow donor site, such as the iliac crest, to precondition resident bone marrow stromal cells (BMSCs) *in vivo*, priming resident MSCs by enlarging and conditioning their population prior to bone marrow aspiration. The resulting sample could then be treated to further augment cell concentration and injected, under fluoroscopic control, into the non-union site through a percutaneous approach.

Introduction

One of the most challenging problems in the orthopedic field is the management of bone non-unions. The incidence of this condition is highly variable depending on site, type of fracture, and whether it is closed or open. The annual incidence of bone fractures in the U.S.A. is approximately 6 million [1] whereas the rate of permanent failure of bone healing is estimated to be 5–10% [2], although other authors report an incidence up to 50% [3]. However, the incidence is believed to be increasing, because of the improved survival rates of patients with multiple injuries [4]. Patients with nonunion can expect more long-term pain, physical disability, mental health problems, higher medical treatment costs, and a slower return to normal work productivity [5]. The economic burden of not healed fractures is relevant because of the cost of the frequently multiple treatments required and the disability associated with the condition. Kanakaris and Giannoudis made a cost identification attempt on a "best-case" scenario in the United Kingdom [6]. They estimated the direct and indirect medical costs at £15,566–17,200 for humeral, tibial, and femoral non-unions. Heckman et al. calculated the cost of treating tibial non-unions in U.S.A. to range from $23,246 to $58,525, depending on the method of treatment provided [7].

Regardless of the surgical treatment adopted, the success rate remains relatively satisfactory with approximately 80% of patients with good to excellent final restoration of mechanical axis alignment and proper length [8]. However, these results include all types of non-unions, and it is realistic to assume that the outcome might be significantly less favorable in the case of atrophic non-unions. Moreover, in the event of further surgery, the rate of success is usually lower. As a consequence, bone regeneration strategies have been added for boosting non-union healing. The current gold standard remains biological autologous bone grafting which, beyond its indisputable effectiveness, has a limited supply, unpredictable reparative potential, requires an additional surgical procedure and is associated with morbidities related to the harvesting procedure [9]. The use of allografts has also been successful, although they are known to undergo resorption and their demand has grown much faster than number of donors. These shortcomings have encouraged the development of artificial scaffolds with the osteoinductive and osteoconductive properties of the natural bone graft and with the capacity of housing osteogenic cells and growth factors. However, these strategies are expensive, technically challenging, and require careful management. Moreover, their value is still uncertain because of the lack of adequate clinical studies necessary to establish their usefulness.

* Corresponding author at: Department of Orthopaedics, Università degli Studi di Milano, Via Festa del Perdono, 7, 20122 Milan, Italy.
E-mail address: valerio.sansone@unimi.it (V. Sansone).

https://doi.org/10.1016/j.mehy.2017.12.013
Received 20 July 2017; Accepted 8 December 2017

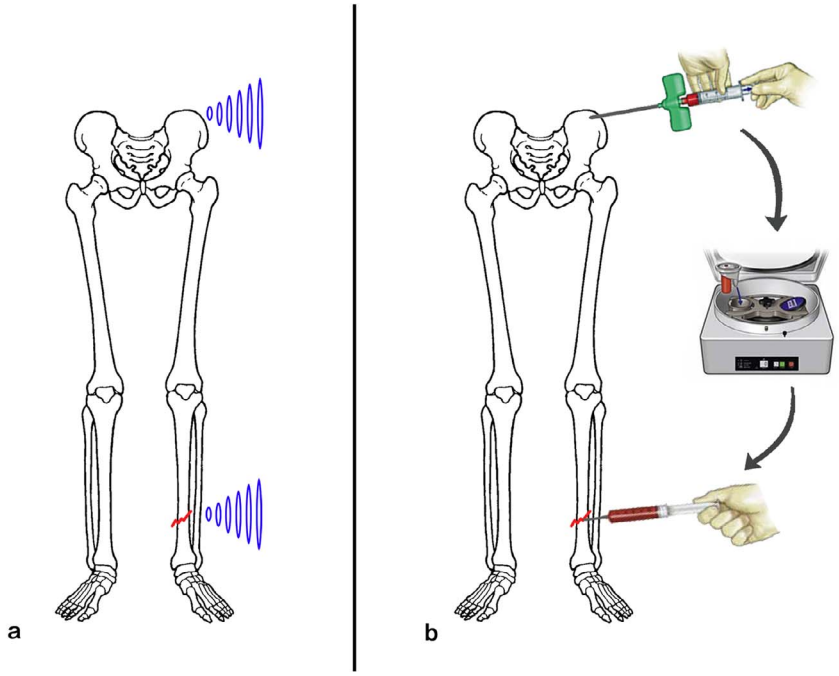

Fig. 1. a) A patient with tibial non-union undergoes 3 sessions of ESWT at the site of non-union and 3 sessions at bone marrow donor site. b) Immediately following the 3rd ESWT session, bone marrow is aspirated from the iliac crest, MSCs are concentrated via density gradient centrifugation, and the resulting BMAC is injected at the site of non-union under fluoroscopic control. International Orthopaedics (SICOT) 2014;38:2585.

Stem cell therapies are gaining momentum in translational research. For example, the use of MSCs via percutaneous bone marrow aspirate concentrate (BMAC) transplants is a potential tool for achieving the goal of augmenting patient biology to promote bone healing [10]. BMAC injections have been shown to be effective in the treatment of non-unions. It has also been demonstrated that the concentration of progenitors was positively correlated to a larger volume of mineralized callus and that greater numbers number colony-forming units lead to faster healing times [11].

Aside from stem cell therapies, ESWT is another conservative technique that has been successfully employed for treating bone non-unions [12–14]. The most relevant findings of many experimental studies indicate that SWs promote MSC growth and differentiation toward osteo-progenitors through Transforming Growth Factor β1 (TGF-β1) and VEGF induction [15,16]. Moreover, recently other mechanisms have been proposed among which Sun and colleagues showed that ESWT induces ATP release and promotes MSC osteogenic differentiation via P2x7 receptors [17]. SWs may also increase the expression of other relevant factors, such as SDF-1 [18–21], although there is not a consensus about the ability of ESWT to regulate SDF-1 expression [22]. Also relevant, SDF-1 it was discovered that SDF-1 is pivotal in the homing and repopulation of MSCs in bone marrow [23].

ESWT in long bone fracture non-unions has been shown to have a success rate ranging from 54% to 98%, depending on the anatomic location of the non-union as well as the elapsed time after the injury and before treatment [24–26]. In light of these results, it has been stated that ESWT is as effective as surgery in achieving healing of long-bone hypertrophic non-unions.[27] However, the results of ESWT in atrophic non-unions are definitively less successful [13,28,29]. Based on this body of evidence, there is an irrefutable need to develop innovative therapies to enhance the bone healing course.

Rationale

The rationale relies on three assumptions:

1. Atrophic non-unions are associated with a deficiency of MSCs at the fracture site [29]. This justifies the transplantation of new cells at the site of injury that can act with two mechanisms [30]:
   a) direct pathway; transplanted cells integrate into the ischemic site and then differentiate in cells specific to the host/homing tissue;
   b) indirect or paracrine pathway; transplanted cells secrete trophic agents and proangiogenic factors that attract resident MSCs to the site of injury and promote angiogenesis, indispensable for tissue reconstruction [31].
2. The number of MSCs in the bone marrow is limited (0.01% of bone marrow cell population) and the results following non-expanded MSC transplantation are unpredictable [32]. As a consequence, current use of MSC therapy often relies on laboratory cultivation and expansion of autologous bone marrow derived MSCs followed by reimplantation at the site of injury. However, cell culture has several drawbacks: the preparation of MSCs is time consuming and it may introduce potential risks; furthermore, therapeutic potency declines with time and with repeated passages in culture [33].
3. SWs are effective in promoting neovascularization and bone healing [19–21] Furthermore, SWs enhance trabecular bone volume and thickness of the treated bone [34] and are able to stimulate biological processes in MSCs, including increased proliferation, survival, and migration [35–37] and to promote osteogenic differentiation of MSCs and of adipose-derived stem cells [38,39]. ESWT may thus represent a potential tool for manipulating MSC behavior for clinical applications.

The hypothesis

Our idea is to the promote healing of atrophic bone non-unions with a combined strategy based on two, concurrent applications of ESWT. One implementation of ESWT would be employed for the well-established biophysical stimulation of the injury site, with the aim of inducing up-regulation and expression of several angiogenic and osteogenic growth factors. A common treatment protocol made of 3 high-energy focal SWs might be suitable. Contemporaneously, another course of ESWT should be delivered at a bone marrow donor site (e.g. the iliac crest) of the patient with the aim of stimulating the bone marrow resident cell population to induce MSC replication and, possibly, their differentiation toward the osteoblastic line. On the same day of the last series of coupled ESWT and after completing the treatments, a bone marrow sample would be harvested from the donor site, centrifuged to increase MSC concentration and then injected under fluoroscopic guidance into the site of pseudoarthrosis (Fig. 1).

The pre-conditioning of the donor site should enlarge the MSC resident population thus significantly increasing the number of cells available for transplantation where their regenerative action is required. The ESWT applied to the injury site would have the aim of "laying the groundwork" for the new cells. Indeed, adequate vascularity is essential for stem cell migration and survival [29,40], especially in the case of atrophic non-unions characterized by a lack of bloody vessels. SWs proved effective in inducing neovascularization through VEGF production in addition to trophic changes achieved through TGFβ1, bone morphogenetic proteins (BMPs), and other bone tissue anabolic growth factors production.

Evaluation of the hypothesis

In order to evaluate the clinical efficacy of a combined treatment protocol consisting of pre-conditioned autologous MSC injection in adjunct to a standard high energy SW treatment protocol, we propose conducting a study with the following characteristics: a group of patients affected by tibial atrophic non-union should be recruited for a prospective, randomized controlled study. An adequate internal fixation of the fracture site must be present. The study group will be randomly divided in 3 sub-groups:

Group 1 – patients will receive 3 ESWT treatments of high energy SWs at the site of non-union at 3 week intervals (treatment 1 and Day 1, treatment 2 at 3 weeks, and treatment 3 at 6 weeks)
Group 2 – patients will receive the same treatment as **group 1** followed by autologous bone marrow aspiration from the iliac crest, concentration via density gradient centrifugation, and injection at the site of pseudoarthrosis.
Group 3 – patients will receive the same treatment as **group 1** plus 3 contemporaneous high energy ESWT treatments on the BMA harvesting site (eg. iliac crest), followed by autologous bone marrow aspiration from the SW treated iliac crest, concentration via density gradient centrifugation, and injection at the site of pseudoarthrosis.

Iliac crest bone marrow is a suitable reservoir of MSCs and thus, for **group 3** patients this area will be repeatedly stimulated with high energy, focused extracorporeal SWs during each of the 3 ESWT treatment sessions. Immediately following the last ESWT session, **group 2** and **group 3** patients will undergo bone marrow aspiration from the anterior iliac crest [41]. Samples will be processed for adequate MSC concentration via density gradient centrifugation. The resulting BMAC will then be injected under fluoroscopic guidance into the site of pseudoarthrosis on the same day [42]. Both the bone marrow aspiration and percutaneous injection into the non-union site will be conducted under IV sedation. **Groups 1** and **2** serve as controls to determine whether the added treatments, either BMAC or ESWT of harvesting site plus BMAC, could be more effective or more rapid in healing atrophic non-unions.

To assess the healing of the fracture, clinical and radiographic criteria will be used. Clinical criteria are based on the ability of the patient to bear full weight on the affected limb and on the absence of pain at the fracture site with manual bending or compression. Imaging assessment includes anteroposterior and lateral radiographs taken at three and six months after treatment. Reestablishment of cortical continuity of a minimum of three of four cortices defines fracture-healing. CT scans will be obtained if the adequacy of fracture-healing cannot be assessed with radiography alone.

Consequences of the hypothesis and discussion

There is a significant clinical need to develop innovative approaches for the treatment of fracture non-unions, mainly of the atrophic forms. MSC-based therapy is a promising tool in the field of regenerative medicine and, in particular, in the treatment of bone defects. In recent years, many research efforts have been made to regenerate bone using stem cells and progenitor cells and our proposal arises as an innovative application of the accumulative conclusions of this recent research. An approach that partially resembles the one we propose, has recently been described by Zhai et al. [43] They used a combined therapy based on repeated courses of SWs at the site of long bone non-union followed by a percutaneous injection of autologous BMAC at the non-union site. Authors report that more than 75% of patients achieved complete healing and the remaining patients showed a marked improvement of the non-union after 12 months from the treatment.

Many studies have shown that physical stimuli have the ability to induce osteogenic lineage commitment in stem cells [44]. We propose the use of ESWT as a non-invasive and effective tool for manipulating MSCs. SWs are an outstanding physical modality that has attracted special attention in the field of *in vivo* tissue regeneration, including bone [34,45], tendon [37,46,47] wounds [48,49], and even the cardiovascular system [50].

It has been shown that the stimulation SWs provide can promote bone healing at a cellular level, inducing proliferation, differentiation, adhesion, and migration of MSCs. Early reports showed that ESWT influences the growth ratio of bone marrow osteoprogenitor cells [13,16,35]. Other experimental studies revealed that SWs can stimulate the osteogenic differentiation and human MSC activity through several molecular pathways, including the regulation of submembrane redox reactions with the activation of extracellular signal-regulated kinases and p38 kinase [14], and focal adhesion kinase [38]. This cascade of events results in the activation of CBFA1 (core-binding factor alpha1) which is the transcription factor for osteoblastic differentiation and osteogenesis. ESWT revealed also able to induce osteogenic differentiation of adipose-derived SCs into osteoblast-like cells [39]. Moreover, in more recent studies, human BMSCs exposed to SWs showed increased proliferation and migration [36].

The hypothesis of preconditioning resident MSCs with the aim of boosting tissue healing through autologous stem cell transplant seems adequately justified by the results of a number of laboratory and clinical studies. This approach appears to have great potential as it suggests that mechanical stress can activate stem cells in their native environment for enhanced therapeutic performance [30,33]. A comparable scenario, although in a different clinical setting, is the successful preventive use of SW in the prophylaxis of wound healing disturbances after vein harvesting for coronary artery by-pass graft surgery [51].

There are at least two other remarkable benefits with this novel procedure. First, there is no need for a laboratory passage for cell expansion, avoiding any genetic manipulation or loss of differentiation capacity and senescence observed with cell culture serial passaging [52]. The second benefit is provided by the method of delivering MSCs via percutaneous injection, substantially reducing morbidity when compared to open surgical implant techniques. The procedure might be repeated if necessary, and it could be used for all the other clinical conditions in which an implemented bone regeneration is requested, mainly in the case of large bone defects.

In conclusion, the herein proposed bimodal application of ESWT employed for expanding MSCs multipotent differentiation capacity as well as their equally central role as cellular modulators, might have the potential to open a new horizon for the treatment of non-unions and bone defects. SW stimulation offers a dynamic approach to manipulating human MSC behavior by exploiting their full regenerative capacity. We close by noting that the same preconditioning model outlined in this paper for the treatment of non-unions, may also be applied in other mesenchymal tissues for different therapeutic needs.

Acknowledgements

The authors would like to thank Rachel Applefield for the illustration in Fig. 1.

References

[1] Bishop JA, Palanca AA, Bellino MJ, Lowenberg DW. Assessment of compromised fracture healing. J Am Acad Orthop Surg 2012;20(5):273–82.
[2] Tzioupis C, Giannoudis PV. Prevalence of long-bone non-unions. Injury 2007;38(Suppl 2):S3–9.
[3] Zimmermann G, Moghaddam A. Trauma: non-union: new trends. Bentley G, editor. European Instructional Lectures, vol 10. Berlin, Heidelberg: Springer; 2010.
[4] Pfeifer R, Tarkin IS, Rocos B, Pape HC. Patterns of mortality and causes of death in polytrauma patients–has anything changed? Injury 2009;40(9):907–11.
[5] Tay WH, de Steiger R, Richardson M, Gruen R, Balogh ZJ. Health outcomes of delayed union and nonunion of femoral and tibial shaft fractures. Injury 2014;45(10):1653–8.
[6] Kanakaris NK, Giannoudis PV. The health economics of the treatment of long-bone non-unions. Injury 2007;38(suppl 2):77–84.
[7] Heckman JD, Sarasohn-Kahn J. The economics of treating tibia fractures. The cost of delayed unions. Bull Hosp Jt Dis 1997;56:63–72.
[8] Rodriguez-Merchan EC, Forriol F. Nonunion: general principles and experimental data. Clin Orthop 2004;419:4–12.
[9] Morshed S, Corrales L, Genant H, Miclau T. Outcome assessment in clinical trials of fracture-healing. J Bone Joint Surg Am 2008;90(suppl 1):62–7.
[10] Hernigou P, Mathieu G, Poignard A, Manicom O, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Surgical technique. J Bone Joint Surg Am 2006;2:322–7.
[11] Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions: influence of the number and concentration of progenitor cells. J Bone Joint Surg Am 2005;87(7):1430–7.
[12] Haupt G. Use of extracorporeal shock waves in the treatment of pseudarthrosis, tendinopathy and other orthopedic diseases. J Urol. 1997;158(1):4–11.
[13] Wang CJ, Chen HS, Chen CE, Yang KD. Treatment of non-unions of long bone fractures with shock waves. Clin Orthop Rel Res 2001;387:95–101.
[14] Chen YJ, Wurtz T, Wang CJ, et al. Recruitment of mesenchymal stem cells and expression of TGF-beta 1 and VEGF in the early stage of shock wave-promoted bone regeneration of segmental defect in rats. J Orthop Res 2004;22(3):526–34.
[15] Wang FS, Wang CJ, Huang HC, Chung H, Chen RF, Yang KD. Physical shock wave mediates membrane hyperpolarization and Ras activation for osteogenesis in human bone marrow stromal cells. Biochem Biophy Res Commun 2001;287:648–55.
[16] Wang FS, Yang KD, Chen RF, Wang CJ, Sheen-Chen SM. Extracorporeal shock wave promotes growth and differentiation of bone-marrow stromal cells towards osteoprogenitors associated with induction of TGF-beta1. J Bone Joint Surg Br 2002;84:457–61.
[17] Sun D, Junger WG, Yuan C, et al. Shockwaves induce osteogenic differentiation of human mesenchymal stem cells through ATP release and activation of P2X7 receptors. Stem Cells 2013;31(6):1170–80.
[18] Aicher A, Heeschen C, Sasaki K, Urbich C, Zeiher AM, Dimmeler S. Low-energy shock wave for enhancing recruitment of endothelial progenitor cells: a new modality to increase efficacy of cell therapy in chronic hind limb ischemia. Circulation 2006;114(25):2823–30.
[19] Tepeköylü C, Wang FS, Kozaryn R, et al. Shock wave treatment induces angiogenesis and mobilizes endogenous CD31/CD34-positive endothelial cells in a hindlimb ischemia model: implications for angiogenesis and vasculogenesis. J Thorac Cardiovasc Surg 2013;146(4):971–8.
[20] Lei PP, Tao SM, Shuai Q, Bao YX, Wang SW, Qu YQ, et al. Extracorporeal cardiac shock wave therapy ameliorates myocardial fibrosis by decreasing the amount of fibrocytes after acute myocardial infarction in pigs. Coron Artery Dis 2013 Sep;24(6):509–15.
[21] Lee JY, Ha KY, Kim JW, Seo JY, Kim YH. Does extracorporeal shock wave introduce alteration of microenvironment in cell therapy for chronic spinal cord injury? Spine 2014;39(26):E1553–9.
[22] Caron J, Michel P, Dussaule J, Chatziantoniou C, Ronco P, Boffa J. Extracorporeal shock wave therapy does not improve hypertensive nephropathy. Physiol Rep 2016;4(11):e12699.
[23] Moll NM, Ransohoff RM. CXCL12 and CXCR4 in bone marrow physiology. Expert Rev Hematol 2010;3(3):315–22.
[24] Schaden W, Fischer A, Sailler A. Extracorporeal shock wave therapy of nonunion or delayed osseous union. Clin Orthop Relat Res 2001;387:90–4.
[25] Elster EA, Stojadinovic A, Forsberg J, Shawen S, Andersen RC, Schaden W. Extracorporeal shock wave therapy for nonunion of the tibia. J Orthop Trauma 2010;24:133–41.
[26] Stojadinovic A, Kyle PB, Eberhardt J, Shawen SB, Andersen RC, Forsberg JA, et al. Development of a prognostic naive bayesian classifier for successful treatment of nonunions. J Bone Jt Surg Am 2011;93:187–94.
[27] Cacchio A, Giordano L, Colafarina O, Rompe JD, Tavernese E, Ioppolo F, et al. Extracorporeal shock-wave therapy compared with surgery for hypertrophic long-bone non-unions. J Bone Joint Surg Am 2009;91(11):2589–97.
[28] Xu ZH, Jiang Q, Chen DY, Xiong J, Shi DQ, Yuan T, et al. Extracorporeal shock wave treatment in non-unions of long bone fractures. Int Orthop 2009;33:789–93.
[29] Tawonsawatruk T, Kelly M, Simpson H. Evaluation of native mesenchymal stem cells from bone marrow and local tissue in an atrophic nonunion model. Tissue Eng Part C Methods 2014;20:524–32.
[30] Zigdon-Giladi H, Rudich U, Michaeli Geller G, Evron A. Recent advances in bone regeneration using adult stem cells. World J Stem Cells 2015;7(3):630–40.
[31] Boregowda SV, Phinney DG. Therapeutic applications of mesenchymal stem cells: current outlook. BioDrugs. 2012;26(4):201–8.
[32] Caplan AI. New era of cell-based orthopedic therapies. Tissue Eng Part B Rev 2009;15:195–200.
[33] Murray IR, Péault B. Q&A: Mesenchymal stem cells: where do they come from and is it important? BMC Biol 2015;13:99.
[34] Tam KF, Cheung WH, Lee KM, Qin L, Leung KS. Shockwave exerts osteogenic effect on osteoporotic bone in an ovariectomized goat model. Ultrasound Med Biol 2009;35:1109–18.
[35] Kearney CJ, Hsu HP, Spector M. The use of extracorporeal shock wave-stimulated periosteal cells for orthotopic bone generation. Tissue Eng: Part A 2012;18(13–14):1500–8.
[36] Suhr F, Delhasse Y, Bungartz G, Schmidt A, Pfannkuche K, Bloch W. Cell biological effects of mechanical stimulations generated by focused extracorporeal shock wave applications on cultured human bone marrow stromal cells. Stem Cell Res 2013;11:951–64.
[37] Brañes J, Contreras HR, Cabello P, Antonic V, Guiloff LJ, Brañes M. Shoulder rotator cuff responses to extracorporeal shockwave therapy: morphological and immunohistochemical analysis. Shoulder & Elbow 2012;4:163–8.
[38] Hu J, Liao H, Ma Z, et al. Focal adhesion kinase signaling mediated the enhancement of osteogenesis of human mesenchymal stem cells induced by extracorporeal shockwave. Sci Rep 2016;6:20875.
[39] Catalano MG, Marano F, Rinella L, et al. Extracorporeal shockwaves (ESWs) enhance the osteogenic medium-induced differentiation of adipose-derived stem cells into osteoblast-like cells. J Tissue Eng Regen Med 2017;11(2):390–9.
[40] Stegen S, van Gastel N, Carmeliet G. Bringing new life to damaged bone: The importance of angiogenesis in bone repair and regeneration. Bone 2015;70:19–27.
[41] Hernigou J, Alves A, Homma Y, Guissou I, Hernigou P. Anatomy of the ilium for bone marrow aspiration: map of sectors and implication for safe trocar placement. Int Orthop 2014;38(12):2585–90.
[42] Jager M, Herten M, Fochtmann U, et al. Bridging the gap: bone marrow aspiration concentrate reduces autologous bone grafting in osseous defects. J Orthop Res 2011 Feb;29(2):173–80.
[43] Zhai L, Ma X-L, Jiang C, Zhang B, Liu S-T, Xing G-Y. Human autologous mesenchymal stem cells with extracorporeal shock wave therapy for nonunion of long bones. Indian J Orthop 2016;50(5):543–50.
[44] Chen JC, Jacobs CR. Mechanically induced osteogenic lineage commitment of stem cells. Stem Cell Res Ther 2013;4(5):107.
[45] Gollwitzer H, Gloeck T, Roessner M, et al. Radial extracorporeal shock wave therapy (rESWT) induces new bone formation in vivo: results of an animal study in rabbits. Ultrasound Med Biol. 2013;39(1):126–33.
[46] de Girolamo L, Stanco D, Galliera E, et al. Soft-focused extracorporeal shock waves increase the expression of tendon-specific markers and the release of anti-inflammatory cytokines in an adherent culture model of primary human tendon cells. Ultrasound Med Biol 2014;40(6):1204–15.
[47] Chao YH, Tsuang YH, Sun JS, et al. Effects of shock waves on tenocyte proliferation and extracellular matrix metabolism. Ultrasound Med Biol 2008;34(5):841–52.
[48] Jeppesen SM, Yderstraede KB, Rasmussen BS, Hanna M, Lund L. Extracorporeal shockwave therapy in the treatment of chronic diabetic foot ulcers: a prospective randomised trial. J Wound Care 2016;25(11):641–9.
[49] Kisch T, Sorg H, Forstmeier V, et al. Remote effects of extracorporeal shock wave therapy on cutaneous microcirculation. J Tissue Viability 2015;24(4):140–5.
[50] Assmus B, Walter DH, Seeger FH, et al. Effect of shock wave-facilitated intracoronary cell therapy on LVEF in patients with chronic heart failure: the CELLWAVE randomized clinical trial. JAMA 2013;309(15):1622–31.
[51] Dumfarth J, Zimpfer D, Vögele-Kadletz M, et al. Prophylactic low-energy shock wave therapy improves wound healing after vein harvesting for coronary artery bypass graft surgery: a prospective, randomized trial. Ann Thorac Surg 2008;86(6):1909–13.
[52] Baxter MA, Wynn RF, Jowitt SN, Wraith JE, Fairbairn LJ, Bellantuono I. Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion. Stem Cells 2004;22:675–82.