CASE REPORT – OPEN ACCESS

International Journal of Surgery Case Reports 34 (2017) 11–16

Contents lists available at ScienceDirect

International Journal of Surgery Case Reports

journal homepage: www.casereports.com

Modified Masquelet technique using allogeneic umbilical cord-derived mesenchymal stem cells for infected non-union femoral shaft fracture with a 12 cm bone defect: A case report

Ismail Hadisoebroto Dilogo[a,d,f,*], Muhammad Rizqi Adhi Primaputra[b], Jeanne Adiwinata Pawitan[c,d,f], Isabella Kurnia Liem[d,e,f]

[a] Consultant of Orthopaedic Trauma and Reconstruction, Department of Orthopaedic and Traumatology, Dr. Cipto Mangunkusumo General Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
[b] Resident of Orthopaedic Surgery, Department of Orthopaedic and Traumatology, Dr. Cipto Mangunkusumo General Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
[c] Department of Histology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
[d] Stem Cell Medical Technology, Integrated Service Unit, Dr. Cipto Mangunkusumo General Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
[e] Department of Anatomy, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
[f] Stem Cell and Tissue Engineering Research Center, Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia

ARTICLE INFO

Article history:
Received 1 December 2016
Received in revised form 1 March 2017
Accepted 3 March 2017
Available online 6 March 2017

Keywords:
Bone defect
Mesenchymal stem cells
Bone morphogenetic protein-2 (BMP-2)
Hydroxyapatite
Masquelet technique
Case report

ABSTRACT

INTRODUCTION: Non-union due to large bone loss often causes significant long-term morbidity. We incorporate the use of allogeneic umbilical cord-derived mesenchymal stem cells (UC-MSCs) as part of the diamond concept of regenerative medicine in a case of infected non-union fracture.
PRESENTATION OF CASE: We reported a 54-year-old female patient presenting with pain on the right thigh. She was previously diagnosed with a closed fracture of the right femoral shaft and underwent four surgeries before finally being referred to Dr. Cipto Mangunkusumo General Hospital with infected non-union of the right femoral shaft. The patient was treated with a combination of UC-MSCs, bone morphogenetic protein-2 (BMP-2), Hydroxyapatite (HA), and mechanical stabilization using Masquelet Technique. The combination of allogeneic MSCs, BMP2, HA, and Masquelet Technique was successful in creating new bone with no apparent side effects.
DISCUSSION: Bone loss might be caused by external factors (true defects), or structural loss of the existing bone. The combination of allogeneic UC-MSCs, BMP-2, HA and an induced membrane technique pioneered by Masquelet allowed for faster regeneration process and more optimal bone healing. This paper aims to assess and compare the result of such procedures with the previous four surgeries done to the patient, which did not yield satisfactory results.
CONCLUSION: The application of allogeneic UC-MSC, BMP-2, HA and Masquelet technique as proposed in the diamond concept is a viable method in treating critical-sized bone defect and provides an effective way to overcome non-union caused by large defect.

1. Introduction

Bone loss often resulted in extended healing period, higher complication rates with its associated hospital costs, and in turn resulted in significant long-term morbidity. The various factors expressed in the diamond concept of regenerative medicine plays an important role in determining bone fracture healing. Moreover, the inoculation of microbial pathogens at the time of initial trauma, during the initial fixation surgery or during the healing process, represent additional factors that may lead to delay of fracture union, loosening of fixation, and chronic osteomyelitis [1–3].

Non-union cases usually involve a complex management strategy, often requiring a number of surgical stages. Recently, Masquelet proposed a simple method of treating bone defect by combining autologous bone grafting that is placed within induced granulation tissue membranes and is applicable to both aseptic and septic conditions. This technique requires no advanced skills in microvascular surgery [1,3,4].

* Corresponding author at: Department of Orthopaedic and Traumatology, Universitas Indonesia, Jln. Diponegoro 71, Jakarta Pusat, 10340, Indonesia.
E-mail address: ismailorthofkui@yahoo.co.id (I.H. Dilogo).

http://dx.doi.org/10.1016/j.ijscr.2017.03.002

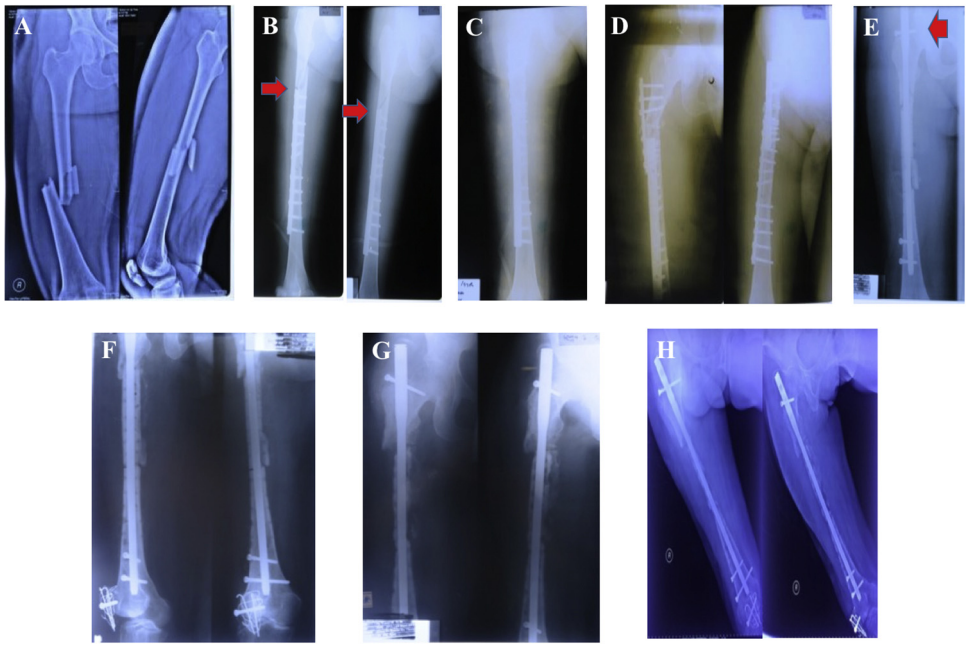

Fig. 1. AP and Lateral Femur X-rays before presenting to our hospital; **A**: Initial presentation; **B**: Post Operative-1 showing missing plate and screw in fixing the proximal fracture above the plating; **C**: Two weeks Post Operative-2 showing another proximal plating; **D**: Post Operative-3 showing proximal plating failure; **E**: Intramedullary nailing conversion showing improper nail insertion and locking fixation; **F**: Femur nailing has been revised and patellar fracture was fixed with screw and wire; **G**: Three months post-operative; **H**: Five months post-operative showing infected and sequestered middle segment of femoral shaft.

We reported a case of 54-year-old female with infected non-union of the right femoral shaft, who underwent a modified Masquelet technique in combination with umbilical cord-derived mesenchymal stem cells (UC-MSCs), bone morphogenetic protein-2 (BMP-2) and bone matrix substitute (Hydroxyapatite (HA), Bongros® -HA, Daewoong), in exchange of the autologous bone graft. This case report represents the application of diamond concept of healing, in line with the SCARE guidelines [5], which was done in Dr. Cipto Mangunkusumo General Hospital, a national top referral academic hospital.

2. Presentation of case

A 54-year-old Indonesian female was referred to Dr. Cipto Mangunkusumo General Hospital with a chief complaint of pain on right thigh since one year ago. One year ago, patient was involved in a motor vehicle accident, and diagnosed with closed fracture of the right femoral shaft (Fig. 1A) and patella. Patient initially presented to a local hospital and underwent open reduction and internal fixation (ORIF) of the femoral fracture using plate and screw. The orthopaedic surgeon opted not to operate on the patella.

Three days after the first operation, patient was informed that the ORIF was insufficient (Fig. 1B) and went for a second revision surgery for another proximal plating. An X-ray was done one-month post-op to evaluate the healing process which showed a new fracture line and proximal plating failure (Fig. 1C). No history of trauma was reported and patient was sent for a third operation for revision of the internal fixation with intramedullary interlocking nail (Fig. 1D).

Two weeks following the third surgery, patient complained of pain during exercise. X-ray showed improper nail insertion and fracture of the patella (Fig. 1E). Revision of intramedullary nailing and tension band wiring was performed for the patellar fracture (Fig. 1F). Unfortunately, three months after the revision surgery, the middle segment of the femoral shaft fracture developed infection and sequestrated (Fig. 1G and H). Patient had an uneventful post-op, was able to walk using axillary crutch and was referred to physiotherapist for range of motion (ROM) exercises. A serous turned purulent discharge was noted at the site of the operation one-month after the final surgery, wound dressing change and antibiotic were prescribed. Patient did not remember the type of antibiotic consumed. Over the next 3 months, no improvement was seen and patient decided to ask for a second opinion.

At the second surgeon, patient was diagnosed with infected non-union femoral shaft fracture with bone defect and was referred to us in Dr. Cipto Mangunkusumo General Hospital. The previous physical examinations and baseline data, including the physiotherapy programs were not available and could not be retrieved at the time of presentation. Patient reported no previous history of diabetes, hypertension or smoking.

On physical examination, a sinus producing purulent discharge from the previous operation scar was noted (Fig. 2). There was pain on palpation, Visual Analog Scale (VAS) of 3–4 and good distal neurovascular function. The knee and hip joint movement was limited due to pain. Lower Extremity Function Scale (LEFS) was calculated to be 27.5% and there was a leg length discrepancy of 5 cm. The discharge was cultured and patient was given oral Cefixime 2 × 200 mg for one month.

From the clinical examination and imaging gathered from previous hospitals, we diagnosed the patient with an infected non-union right femoral shaft fracture with significant bone loss and was planned for a modified Masquelet Technique.

The modified Masquelet Technique for treatment of segmental bone defects with the addition of UC-MSCs was done (Fig. 3A and B). The operation began with debridement, removal of sinus tract and intramedullary nail, followed by bone cement spacer nailing insertion and coil wire. The surgery was performed by a senior orthopaedic surgeon specializing in trauma and adult reconstruction. Patient was positioned in left lateral decubitus position under spinal anesthesia. Incision was made on the precious scar. Sinus discharge was collected and sent for culture. A sequestrum from the femoral bone was removed and debrided. Intramedullary nail was removed from the tip of the femur followed by flushing of the femur using saline and hydrogen peroxide. A bone spacer consisting

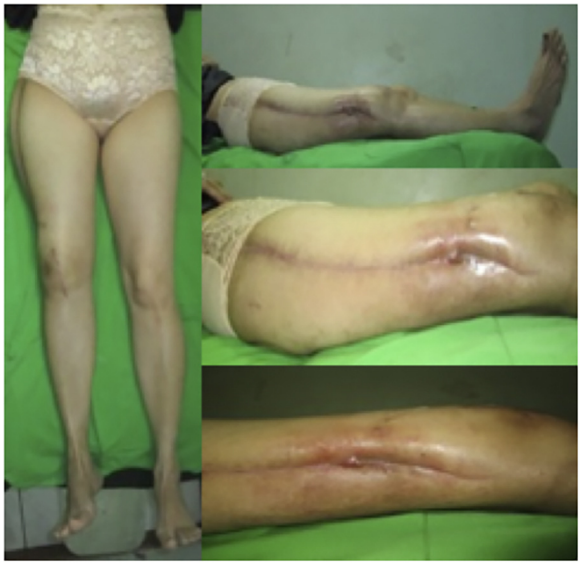

Fig. 2. Clinical presentation of the patient. Note the presence of a purulent discharge producing sinus at initial presentation.

of coil wire with a diameter of 1.5 mm, was aligned parallel to the femur and was inserted in the intramedular femur for a distance of 20 cm. Bone cement containing gentamicin and vancomycin antibiotic was also added. The culture of the sinus tract discharge showed infection caused by *Escherichia coli* and *Staphylococcus epidermidis*. The patient did not receive any physiotherapy after the first surgery.

Two months after surgery, patient underwent a second procedure consisting of debridement, spacer removal and ORIF using reverse distal femoral locking plate with 13 holes (Synthes). A 12 cm bone defect was noted after spacer removal (Fig. 4A and B). We filled the bone defect using bone substitute material containing Hydroxyapatite (Bongros®-HA, Daewoong) along with BMP-2 and 50 million UC-MSCs to enhance bone regeneration.

The UC-MSCs were isolated from an umbilical cord, obtained from a healthy donor who delivered by Caesarean section, using multiple harvest explants method [6], cryopreserved in passage 3, and stored in Stem Cell Medical Technology, Integrated Service Unit, Dr. Cipto Mangunkusumo General Hospital. Before implantation, the cryopreserved MSCs were thawed, and recultured in in-house developed medium, and were characterized as MSCs, according to International Society for Cellular Therapy (ISCT) [7]. All procedures were done in a cGMP facility. The UC-MSCs were suspended in 10 mL of patient serum.

There were no complications and adverse events reported postoperatively. Patient was followed up for up to 12 months and clinical evaluation and radiographic imaging were taken (Fig. 5). Patient underwent rehabilitation using 12 cycle of Transcutaneous Electrical Nerve Stimulation (TENS) and 6 cycle of heat therapy at our Rehabilitation Center. Patient was instructed to mobilize using bilateral axillary crutches until there is evidence of clinical union. At 6 months post-op, patient exhibited clinical union and started to walk using crutch on the right side (Fig. 6A). Patient reported no pain (VAS: 0) with walking and clinical examination still shows a leg length discrepancy of 2 cm. LEFS showed improvement at 30%.

At 8-month follow up, patient complained of discomfort while moving her knee joint and decided to have the patellar implant removed.

By the 11-month follow up period, clinical examination showed that patient was able to perform full weight bearing walk (Fig. 6B) with no pain (VAS: 0), LEFS of 65%, with no change in leg length discrepancy. The scar was clean, with no wound dehiscence and no signs of infection.

3. Discussion

Bone loss is a widely used term referring to structural defects and regions of missing bone caused by external factors (true defects) and structural loss within existing bone, such as in osteopenia. Primary bone loss can occur in primary bone diseases, such as bone fracture due to trauma or malignancy where bone destruction can lead to bone loss, whereas secondary bone loss is most commonly due to external factors such as infection or tumor resection [2,8].

In an acute setting, trauma is considered to be the major cause of bone loss. Following trauma to bone tissue, late complication such as non-union can occur when bone fails to heal, particularly if the patient has previous underlying conditions that may impair healing [2,9,10]. In our case, we found no underlying conditions, such as diabetes or history of smoking, that may cause failure of bone to heal. We proposed that the failure was due the instability of the previous internal fixation.

The main things to consider for any reconstruction plans are the quality of soft tissue envelope, vascular supply and the presence or absence of infection [10]. There are many available treatments, particularly for large defects but most recently, a two-stage induced membrane technique pioneered by Masquelet and colleagues has received extensive attention [11–13].

Masquelet proposed a procedure combining induced membranes and cancellous autografts for the reconstruction of extensive diaphyseal bone loss, without the need for vascularized autograft. These allows for the reconstruction of wide diaphyseal defects,

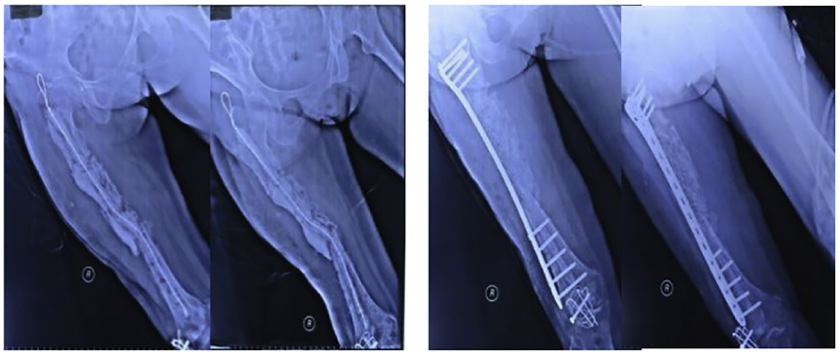

Fig. 3. Modified Masquelet Technique; **A**: Insertion of bone cement spacer after debridement and middle segment sequester was removed (Masquelet Stage I), **B**: Bone cement spacer was removed and femoral fracture was fixed by reverse distal femur LCP and middle segment bone defect was filled with HA granules, BMP-2 and allogeneic UC-MSCs (modified Masquelet Stage II).

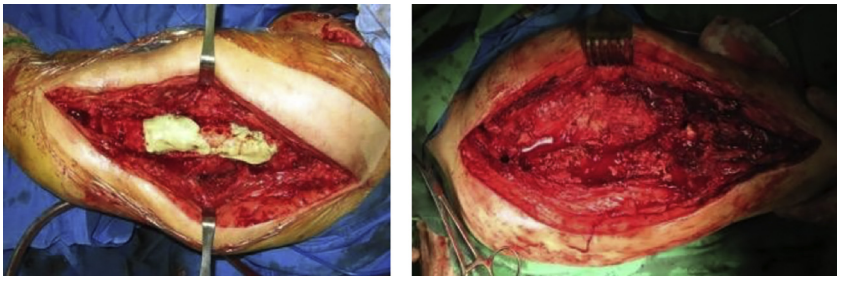

Fig. 4. Exposure of bone defect before and after removal of spacer.

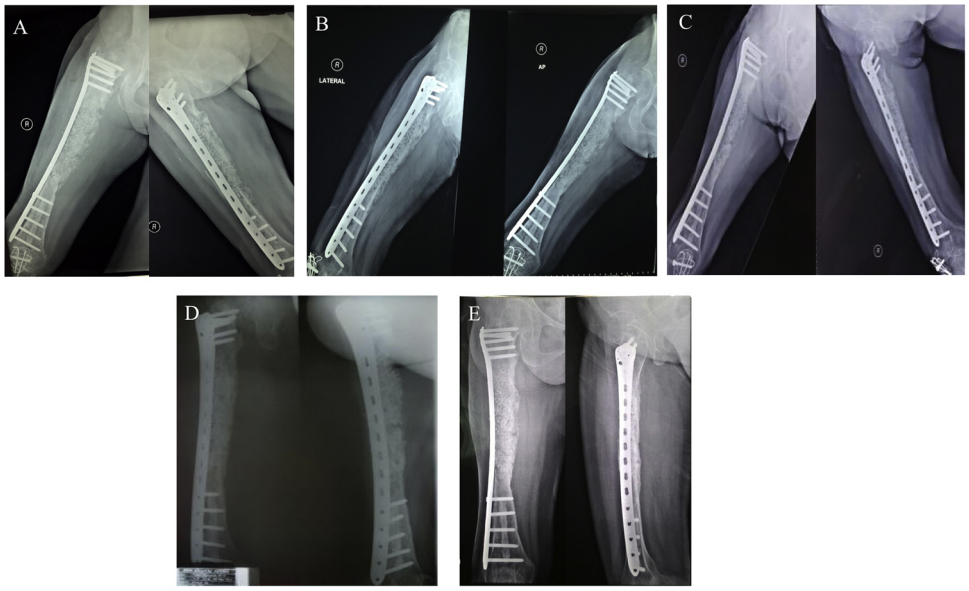

Fig. 5. Post-Operative follow up AP and Lateral Femur X-Rays; A: 1 month; B: 3 months; C: 6 months; D: 9 months; E: 12 months.

even in cases where the recipient site has been irradiated or infected, provided that an envelope is previously created to protect and revascularized the bone graft [11,14,15].

The Masquelet surgical procedure consisted of two steps. In the first step, a thorough debridement is performed, the segmental bone defect is bridged using a polymethylmethacrylate (PMMA) cement spacer and the bone is stabilized using orthopaedic hardwares. In a grossly contaminated or infected wounds, more than one surgical debridement and temporary stabilization may be required [4,11,15,16].

The spacer induces the formation of encapsulating thin fibrous membrane made of type 1 collagen-heavy matrix with fibroblastic cells and contains high concentrations of growth and osteogenic factors. After 4–8 weeks, the membrane is incised and the spacer removed while still preserving the membrane. The leftover cavity is filled with autologous cancellous graft harvested from iliac crest or femoral canal (with or without the addition of allograft) and the membrane slit is then closed. Union is usually achieved within 8.5 months, with the patients recovering to normal gait and motion. We consider our procedure to be a modified Masquelet technique due to the fact that we use UC-MSCs implantation as a replacement of autogenous and synthetic bone graft. Current studies suggest that the induced membrane plays a role in osteogenesis and vascularization of bone graft [4,6,8,10,12,14–16]. In our case, clinical union was achieved at 6 months post operatively and patient was able to mobilize with the help of axillary crutches. Abnormal gait was still present due to leg length discrepancy.

In this particular case, in addition to bone substitutes, we also add in UC-MSCs and BMP-2 to enhance the regeneration process.

Fig. 6. A: 6-month post-operative. Patient walked with partial weight bearing using crutches. B: 11-month post-operative. Patient walked with full weight bearing.

The basic principle of regeneration in bone tissue engineering also known as the diamond concept consists of potent osteogenic cell populations, osteoinductive stimulus, osteoconductive matrix scaffolds and mechanical environment [17–19].

We used allogeneic UC-MSCs in addition to the natural osteogenic population of patient's own bone to serve as the

osteogenic cell populations. MSCs are stem cells of mesenchymal origin and are undifferentiated cells with high proliferative capability, capable of self-renewal, multi-lineage differentiation and tissue regeneration. However, MSCs have varying degrees of differentiation potential and interaction with other cell types and components of the extracellular matrix are believed to influence the survival and development of MSCs to the committed lineage [20].

MSCs have been widely used for bone tissue engineering because they are free of both ethical concerns and teratoma risk that exists with using Embryonic Stem Cells. MSCs are obtained easily from almost every tissue in the human body and are easy to culture in both *in vitro* and *in vivo* study. MSCs do not express major histocompatibility complex (MHC) class II markers and several studies suggest that they are relatively immune-privileged and may be used as allografts with the risk of rejection [20–22].

In an acute setting, such as in fracture cases, osteogenic stimuli originated from a number of cytokines and growth factors secreted by endothelial cells, platelets, macrophages, monocytes, MSCs, chondrocytes, osteocytes and osteoblasts. They may induce a cascade of cellular events that initiate healing. BMP-2 and BMP-7 are members of the Transforming Growth Factor β (TGF-β) superfamily that have been studied extensively are their applications in biological healing enhancement at areas of delayed fracture healing or nonunion have been established [18,20].

Extracellular matrix serves as a natural scaffold for cellular events and interactions of various biologic substances. In bone tissue engineering, porous biomaterials such as allograft or xenograft trabecular bone, Demineralized Bone Matrix (DBM), hydroxyapatite and many others have been studied extensively for their utility as bone scaffold. They can be used alone or in combination with bone active growth factors in an attempt to achieve a maximum osteogenic effect as bone defect filler [17,18,20].

Recent trend towards osteosynthesis is to give relative stability and maximal respect for soft tissue envelope and the vascularity around the fracture site. Splints, cases, intramedullary nails, external fixators and locking plates stabilize the fracture site by minimizing the interfragmentary gap size and keeping the interfragmentary strain below 10%. However, in the setting of fracture fixation in conjunction with bone grafting, the mechanical stability necessary for optimal healing has not been adequately studied. The general consensus is that a certain load-shielding period has to be achieved to protect the graft in its initial incorporation phase [18].

To our knowledge, this is the first case report combining Masquelet technique with UC-MSCs, BMP-2 and HA. Each represents one of the four pillars of the diamond concept, namely osteogenic, osteoinductive, osteoconductive and stable fixation. The implanted exogenous UC-MSCs may be able to stimulate dormant MSCs in proximal and distal segment of femoral shaft fracture through paracrine effect. UC-MSCs may also have a direct effect by proliferation and differentiation of MSCs into osteogenic cells. UC-MSCs have been shown to survive in HA and directed its effect on osteogenic cells which are additionally induced by exogenous BMP-2. Moreover, the presence of growth factors within biological induced membrane that is created by the Masquelet technique may play a part in the healing process.

This report also favors UC-MSCs-based therapy for critical-sized bone defect. This readily available therapy may provide an alternative method in dealing with the disadvantages and weaknesses of the non-vascularized or vascularized fibular bone graft and bone transports surgery that most often resulting in joint stiffness and requires exceptional patient compliance during distraction osteogenesis and rehabilitation period. This procedure showed that there were significant bone consolidation and clinical improvement in less than 1 year of follow up.

4. Conclusions

In summary, this is a case of an infected non-union fracture case that was difficult to treat by means of previously mentioned surgical methods. We performed a modified Masquelet technique in combination with allogeneic UC-MSCs, HA granules and BMP-2. We showed that this modified technique is an effective way to overcome large bone defects and the addition of allogeneic UC-MSCs and BMP-2 delivers growth and osteoinductive factors as well as enhances healing of the defected bone, in accordance with the diamond concept.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Funding

This research received grant from the Program Insentif Riset Sistem Inovasi Nasional (INSINAS) National Innovation System Research Incentive (INSINAS) 2016.

Ethical approval

Ethical approval from the Health Research Ethics Committee Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo General Hospital.

Reference number: 165/H2.F1/ETIK/2014.

Consent

Written informed consent was obtained from the patient and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Author contribution

IHD, MRAP – examining patient, following up patient, writing up the manuscript and reviewing the literature.

IHD – senior orthopaedic surgeon assigned to the case.

JAP and IKL – culture of the UC-MSCs and production of BMP-2.

Registration of research studies

Clinical Trial Registry Number: NCT 0172 5698.

Guarantor

Ismail Hadisoebroto Dilogo, M.D., PhD. (corresponding author) Orthopaedic Surgeon, Hip and Knee Consultant at Department of Orthopaedic and Traumatology, Faculty of Medicine Universitas Indonesia and head of Stem Cell Medical Technology, Integrated Service Unit, Dr. Cipto Mangunkusumo General Hospital, Faculty of Medicine Universitas Indonesia.

Acknowledgement

We acknowledge everyone at Stem Cell Medical Technology, Integrated Service Unit, Dr. Cipto Mangunkusumo General Hospital, Faculty of Medicine Universitas Indonesia namely Tri Kurniawati, Tera Kispa, Fajar Mujadid and Novialdi for their continuous support and assistance.

References

[1] T.A.J. Goff, N.K. Kanakaris, Management of infected non-union of the proximal femur: a combination of therapeutic techniques, Injury 45 (2014) 2101–2105, http://dx.doi.org/10.1016/j.injury.2014.08.046.
[2] A. Wiese, H.C. Pape, Bone defects caused by high-energy injuries, bone loss, infected nonunions, and nonunions, Orthop. Clin. North Am. 41 (2010) 1–4, http://dx.doi.org/10.1016/j.ocl.2009.07.003.
[3] T.M. Wong, T.W. Lau, X. Li, C. Fang, K. Yeung, F. Leung, Masquelet technique for treatment of posttraumatic bone defects, Sci. World J. 2014 (2014) 710302, http://dx.doi.org/10.1155/2014/710302.
[4] A.J. Micev, D.M. Kalainov, A.P. Soneru, Masquelet technique for treatment of segmental bone loss in the upper extremity, J. Hand Surg. Am. 40 (2015) 593–598, http://dx.doi.org/10.1016/j.jhsa.2014.12.007.
[5] R.A. Agha, A.J. Fowler, A. Saeta, I. Barai, S. Rajmohan, D.P. Orgill, The SCARE statement: consensus-based surgical case report guidelines, Int. J. Surg. 34 (2016) 180–186, http://dx.doi.org/10.1016/j.ijsu.2016.08.014.
[6] J.A. Pawitan, I.K. Liem, E. Budiyanti, I. Fasha, L. Feroniasanti, T. Jamaan, K. Sumapradja, Umbilical cord derived stem cell culture: multiple-Harvest explant method, Int. J. PharmTech Res. 6 (2014) 1202–1208.
[7] E. Budiyanti, I.K. Liem, J.A. Pawitan, D. Wulandari, T. Jamaan, K. Sumapradja, Umbilical cord derived mesenchymal stem cell proliferation in various platelet rich plasma and xeno-material containing medium, Int. J. Res. Pharm. Sci. 6 (2015) 7–13.
[8] D. Smrke, P. Rožman, M. Veselko, B. Gubina, Treatment of bone defects – allogenic platelet gel and autologous bone technique, in: J.A. Andrades (Ed.), Regen. Med. Tissue Eng., InTech, 2013, http://dx.doi.org/10.5772/55987.
[9] P.V. Giannoudis, Treatment of bone defects: bone transport or the induced membrane technique? Injury 47 (2016) 291–292, http://dx.doi.org/10.1016/j.injury.2016.01.023.
[10] H.C. Pape, T. Pufe, Bone defects and nonunions—what role does vascularity play in filling the gap? Injury 41 (2010) 553–554, http://dx.doi.org/10.1016/j.injury.2010.04.001.
[11] B.M. Adamova, S. Vohanka, M. Hnojcikova, I. Okacova, L. Dusek, J. Bednarik, Neurological impairment score in lumbar spinal stenosis, Eur. Spine J. 22 (2013) 1897–1906, http://dx.doi.org/10.1007/s00586-013-2731-7.
[12] N.G. Lasanianos, N.K. Kanakaris, P.V. Giannoudis, Current management of long bone large segmental defects, Orthop. Trauma. 24 (2010) 149–163, http://dx.doi.org/10.1016/j.mporth.2009.10.003.
[13] R. Gouron, F. Deroussen, M.C. Plancq, L.M. Collet, Bone defect reconstruction in children using the induced membrane technique: a series of 14 cases, Orthop. Traumatol. Surg. Res. 99 (2013) 837–843, http://dx.doi.org/10.1016/j.otsr.2013.05.005.
[14] P.V. Giannoudis, O. Faour, T. Goff, N. Kanakaris, R. Dimitriou, Masquelet technique for the treatment of bone defects: tips-tricks and future directions, Injury 42 (2011) 591–598, http://dx.doi.org/10.1016/j.injury.2011.03.036.
[15] V. Viateau, M. Bensidhoum, G. Guillemin, H. Petite, D. Hannouche, F. Anagnostou, P. Pélissier, Use of the induced membrane technique for bone tissue engineering purposes: animal studies, Orthop. Clin. North Am. 41 (2010) 49–56, http://dx.doi.org/10.1016/j.ocl.2009.07.010.
[16] C. Nau, C. Seebach, A. Trumm, A. Schaible, K. Kontradowitz, S. Meier, H. Buechner, I. Marzi, D. Henrich, Alteration of Masquelet's induced membrane characteristics by different kinds of antibiotic enriched bone cement in a critical size defect model in the rat's femur, Injury 47 (2016) 325–334, http://dx.doi.org/10.1016/j.injury.2015.10.079.
[17] J.C. Aurégan, T. Bégué, Induced membrane for treatment of critical sized bone defect: a review of experimental and clinical experiences, Int. Orthop. 38 (2014) 1971–1978, http://dx.doi.org/10.1007/s00264-014-2422-y.
[18] P.V. Giannoudis, T.A. Einhorn, D. Marsh, Fracture healing: the diamond concept, Injury 38 (2007), http://dx.doi.org/10.1016/s0020-1383(08)70003-2.
[19] I.H. Dilogo, A.F. Kamal, B. Gunawan, R.V. Rawung, Autologous mesenchymal stem cell (MSCs) transplantation for critical-sized bone defect following a wide excision of osteofibrous dysplasia, Int. J. Surg. Case Rep. 17 (2015) 106–111, http://dx.doi.org/10.1016/j.ijscr.2015.10.040.
[20] A.J. Salgado, O.P. Coutinho, R.L. Reis, Bone tissue engineering: state of the art and future trends, Macromol. Biosci. 4 (2004) 743–765, http://dx.doi.org/10.1002/mabi.200400026.
[21] A.J. O'Connor, W.A. Morrison, Tissue engineering, Plast Surg. (2013) 367–396.
[22] X. Wei, X. Yang, Z. Han, F. Qu, L. Shao, Y. Shi, Mesenchymal stem cells: a new trend for cell therapy, Acta Pharmacol. Sin. 34 (2013) 747–754, http://dx.doi.org/10.1038/aps.2013.50.