ORIGINAL ARTICLE

Allogeneic umbilical cord-derived mesenchymal stem cells for treating critical-sized bone defects: a translational study

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Abstract

Introduction The current 'gold-standard' treatment of critical-sized bone defects (CSBDs) is autografts; however, they have drawbacks including lack of massive bone source donor site morbidity, incomplete remodeling, and the risk of infection. One potential treatment for treating CSBDs is bone marrow-derived mesenchymal stem cells (BM-MSCs). Previously, there were no studies regarding the use of human umbilical cord-mesenchymal stem cells (hUC-MSCs) for treating BDs. We aim to investigate the use of allogeneic hUC-MSCs for treating CSBDs.

Method We included subjects who were diagnosed with non-union fracture with CSBDs who agreed to undergo hUC-MSCs implantation. All patients were given allogeneic hUC-MSCs. All MSCs were obtained and cultured using the multiple-harvest explant method. Subjects were evaluated functionally using the Lower Extremity Functional Scale (LEFS) and radiologically by volume defect reduction.

Result A total of seven (3 male, 4 female) subjects were recruited for this study. The subjects age ranged from 14 to 62 years. All seven subjects had increased LEFS during the end of the follow-up period, indicating improved functional ability. The follow-up period ranged from 12 to 36 months. One subject had wound dehiscence and infection, and two subjects developed partial union.

Conclusion Umbilical cord mesenchymal stem cells are a potential new treatment for CSBDs. Additional studies with larger samples and control groups are required to further investigate the safety and efficacy of umbilical cord-derived mesenchymal stem cells for treating CSBDs.

Keywords Critical-sized bone defect · Umbilical cord · Mesenchymal stem cells

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Introduction

Critical-sized bone defects (CSBDs) are commonly found in various clinical settings, such as congenital malformation, cancer, trauma, and infection [1, 2]. CSBDs present significant challenges in treating orthopedic disorders, as bones have limited intrinsic regeneration capacity [3]. Currently, the gold-standard treatment for CSBDs is the implantation of autologous bone grafts, as they have the osteogenic, osteoconductive, and osteoinductive characteristics required to heal such bone defects. However, this method faces challenges like the lack of massive bone source, donor site morbidity, incomplete bone remodeling, and risk of infection [1, 2, 4, 5]. Less preferred methods include allogenic bone grafts and xenografts; however, these treatments often lack osteogenic properties, making them ineffective. This highlights the increasing need for another therapeutic strategy

that is affordable, atraumatic, and safe bone grafting strategies [1, 2].

The solutions to the aforementioned problems may be given by multidisciplinary field such as tissue engineering (TE), which attempts to combine cells with scaffolds and growth factors in order to create bio-artificial tissues [2]. Mesenchymal stem cells (MSCs), self-renewing multipotent progenitor cells that can be isolated from adult and fetal tissues [6], have garnered interest due to their potential for enhancing bone TE. These cells are able to differentiate into various cell lineages including bone cells [7, 8], allowing them to regenerate damaged and diseased bone. Currently, the gold standard of bone and cartilage TE research remains bone marrow-derived MSCs (BM-MSCs), which are multipotent and able to differentiate into various cells including osteoblasts, chondrocytes, neurons, myoblasts, adipocytes, and fibroblasts. These cells can be harvested from bone marrow, expanded in culture, induced to differentiate, and combined with a scaffold to repair CSBDs [9].

Despite being able to repair CSBDs, BM-MSCs have several limitations such as donor-site morbidity, low yield in sourcing, difficulty in expansion in vitro, and infection, and a declining differentiation potential inversely proportional to their age [2, 10, 11]. Those limitations could be potentially overcome with the use of another type of MSC from human umbilical cord (UC). Previously, there were no studies regarding the use of hUC-MSCs for treating CSBDs. This study aims to investigate the use of allogeneic hUC-MSCs for treating CSBDs.

Methods

All patients were given allogeneic hUC-MSCs. Umbilical cords were taken from mothers who had elective full-term cesarean section without complications. All samples were screened for HIV, hepatitis B, hepatitis C, fungal, and bacterial contamination. All MSCs were obtained and cultured using the multiple-harvest explant method, which was established by Pawitan et al. [12]

We included subjects age 14–62 years with CSBD who agreed to undergo the hUC-MSC implantation. Patients with diabetes mellitus, cardiovascular disease, autoimmune disease, history of pathologic fracture, ongoing hormonal therapy and active osteomyelitis or soft tissue infection were excluded from this study.

The Masquelet technique of implantation was performed in two stages. The first stage involved radical debridement of all infected or non-viable bone and interposed fibrous tissue. Following debridement, stabilization by plating internal fixation was achieved to maintain length, alignment, and rotation prior to insertion of the cement spacer [13].

Procedure

Ten centimeters of UC was collected in 50 mL transport medium, which contained alpha minimal essential medium (αMEM, GIBCO 41061-029), 1% penicillin/streptomycin (GIBCO 15140-122), 1% amphotericin B (GIBCO 15290-026), 1% L-glutamax (GIBCO 15050-061), and 10% platelet lysate.

Cells of passage 3–6 were used. The cells met the requirement of the International Society for Cell Therapy (ISCT), in terms of adherence to plastics, morphology, surface markers, and three-lineage differentiation capacity. Sterility checks were performed three times, in the beginning, mid, and end of culture. Only cells that were proven sterile through these sterility checks were given to patients as part of their treatment.

The subjects underwent two stages of Masquelet technique. In the first step, a thorough debridement was performed. The margins of the bone defect were cut until the medullary cavity and bone marrow was reached, and the intramedullary canals were drilled for a certain distance to increase vascularity. The segmental bone defect was bridged by polymethylmethacrylate (PMMA) cement spacer, and the bone was stabilized with plate and screw. In cases of severe contamination or infection, multiple surgical debridement and temporary stabilization procedures were performed before definitive fixation and insertion of a cement spacer was conducted.

The spacer served to induce the formation of encapsulating thin fibrous membrane. Eight weeks following insertion of the spacer, the second step of the Masquelet technique was performed. The fibrous membrane was incised, the spacer was removed, and cavity was then filled with osteogenic, osteoinductive, and osteoconductive agents.

Firstly, bone cement spacer was removed, and bone defect site was prepared. For the osteoconductive agents, 5 g of hydroxyapatite (HA) granules (Bongros®-HA, Daewoong) was filled with 50 million hUC-MSCs to fill the bone gap. 1.3 ml BMP-2 (Novosis®, Daewoong, Ltd) per 5 g HA granules which has been proven to enhance MSC differentiation was added as an osteoinductive agent and finally sealed with demineralized bone matrix (Bonegener®, Daewoong, Ltd).

Patients were observed for 6 and 12 months following operation. Outcomes were observed by clinical and radiological examinations. The functional outcomes were observed using the LEFS score (Lower Extremity Functional Scale score) for lower extremity defects. Callus formation was observed through serial X-ray examinations, and defect volume was calculated using the following formula from two projections of plain radiograph:

$$V = L_{AA1} \times H_{AA1} \times W_{AA2}$$

$V =$ volume, $L =$ length, $W =$ width, $AA1 =$ the first projection of plain radiograph, $AA2 =$ the second projection of plain radiograph.

Result

A total of seven (3 male, 4 female) subjects were recruited for this study. The age ranged from 14 to 57 years. All seven subjects showed increased LEFS during the end of the follow-up period, indicating improved functional ability. The follow-up period ranged from 12 to 36 months (Table 1). One subject had wound dehiscence and surgical site infection, and two subjects developed partial non-union (Table 2). Radiography of Case 1, Case 2, and Case 3 is provided in Figs. 1, 2, and 4. One of patients who developed partial non-union (Subject 3) had high final volume defect. This condition happened due to insufficient soft tissue coverage to close the surgical site due to skin loss; therefore, the entirety of tibial shaft could no longer unite. To overcome this condition, the surgeon made a union crossing between tibia and fibula; therefore, patient can perform weight-bearing activities (Fig. 3).

Discussion

There are various approaches by which critical-sized bone defects are being treated, such as bone grafting, distraction osteogenesis, and induced membrane (Masquelet) technique [14]. Nowadays, surgeons may opt to use various types of bone grafts in accordance with the size of defects, including cancellous autograft, allografts, and vascularized fibular grafts; however, each type of grafts comes with each advantages and setbacks. Cancellous autograft may give more desired result in smaller defects (<5 cm), whereas larger defects could yield more benefit from allografts and vascularized fibular grafts. Nevertheless, these grafts lack of osteogenic properties and need MSCs supplementation to stimulate bone regeneration [14, 15].

Mesenchymal stem cells (MSCs), also known as marrow stromal cells or mesenchymal progenitor cells, are defined as self-renewal, undifferentiated cells with the capacity to differentiate into several distinct mesenchymal lineages. To date, MSCs of multiple adult vertebrate species have been demonstrated to differentiate into connective skeletal tissue, bone, cartilage, osteoblasts, chondrocytes, marrow stroma and adipocytes, making their usage possible in the orthopedic field [16, 17]. The primary sources of MSCs for clinical use are BM and adipose tissue (AT); however, their utility is limited by the need for invasive harvesting procedures and strict donor age requirements (Fig. 4). The frequency and differentiating capacity of MSCs also decrease with donor

Table 1 Characteristics of the subjects

| Initial	Subject	Age (years) at MSC injection	Sex	Bone	Cause	Procedure	Initial LEFS score (%)	Final LEFS score (%)	Volume defect (cm³)	Final volume defect (cm³)	Follow-up (months)	Initial VAS	Final VAS
YHa	1	57	F	Femur	Trauma	Masquelet, ORIF plate screw	27.5	70	50	0	36	0	0
AAZ	2a*	16	M	Femur	Trauma	Masquelet, bone graft, ORIF revision, plate screw	43.75	65	24	0	13	0	2
AAZ	2b*	16	M	Tibia	Trauma	Masquelet, bone graft, ORIF plate screw	43.75	65	43,88	0	12	0	2
DAr	3	22	M	Tibia	Trauma	Masquelet	36.25	46.25	46	41**	18	0	0
DSi	4	62	F	Right femur	Trauma	Masquelet, ORIF plate screw	47.5	60	2	0	21	2	2
ATe	5	39	F	Femur	Trauma	Masquelet, ORIF PS, bone graft	7.5	50	50	0	21	5	5
AAS	6	14	F	Femur	Trauma	Masquelet, ORIF PS, bone graft	18.75	36.25	9	0	15	0	0
Suy	7	52	M	Femur	Trauma	Masquelet, ORIF PS, bone graft	41.25	61.25	41.2	3.3	30	3	3

BD bone defect, F female, MSC mesenchymal stem cell, LEFS Lower Extremity Functional Scale, M male, ORIF open reduction internal fixation

*One subject had two fracture sites, and analysis was carried out according to fracture site

**The cause of high final volume defect is due to insufficient soft tissue coverage to close the surgical site due to skin loss; therefore, the tibia could no longer grow and eventually developed partial non-union

Table 2 Complications that occurred in the subjects

| Subject	Leg length discrepancy (cm)	Complications	Surgical revision	Follow-up (months)
2b	0	Wound dehiscence, surgical site infection	Implant removal	13
3	4	Partial union (union between the tibia and fibula)	Leg-lengthening procedure	18
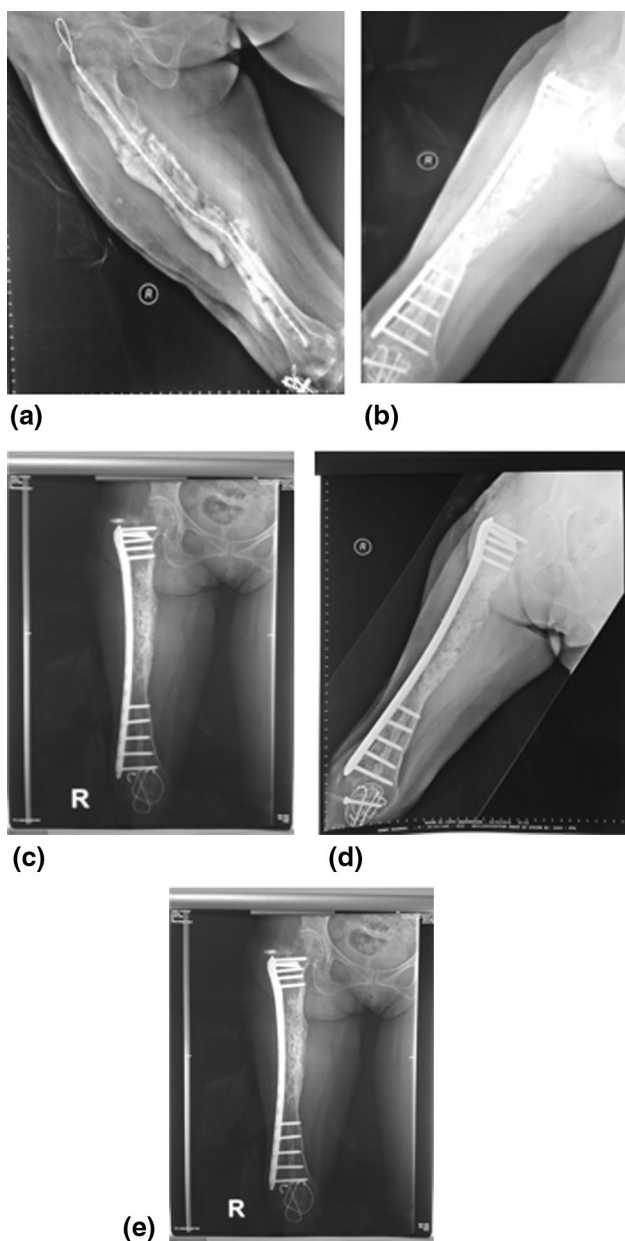
Fig. 1 Radiography of Subject 1. **a** Preoperative femoral radiography. **b** Postoperative femoral radiography. **c** 6-month postoperative radiography. **d** 12-month postoperative radiography. **e** 29-month postoperative radiography

age; thus, cells that derived from elderly patients may be clinically ineffective [17, 18]. Due to these limitations, alternative sources of MSCs have been sought (Fig. 5).

Implanted exogenous hUC-MSCs may be able to stimulate dormant MSCs in the fractures through a paracrine effect [19]. hUC-MSCs may also have a direct effect by the proliferation and differentiation of MSCs into osteogenic cells [20]. In this study, we use allogeneic hUC-MSCs to treat CSBDs. As multipotent cells, hUC-MSCs can differentiate into osteogenic and chondrogenic lineages, allowing them to regenerate bone. This makes hUC-MSCs an attractive potential alternative to bone grafts. Moreover, using hUC-MSCs can avoid ethical and technical issues [18], as they are obtained by a simple, safe, and painless procedure when a baby is delivered. Although allografts are currently used for a variety of orthopedic procedures, they are expensive and carry the risk of viral infection and immunorejection associated with poor biological and mechanical properties [2].

Due to their osteogenic potential and immunomodulatory, anti-inflammatory, and antiapoptotic properties, MSCs have the potential to be used as the primary treatment for diseases affecting bone tissues [21]. Initially, the focus of MSCs treatment of musculoskeletal injuries was based on their ability to differentiate into several cell types [21–24]. It was expected that upon implantation, the cells would colonize and differentiate at the lesion site along the appropriate MSCs lineage. However, it is found that the survival and differentiation of MSCs at the site of the lesion is limited [25] and that the primary benefit of MSCs is in their paracrine signaling on neighboring cells.[26] The molecules secreted, such as growth factors, cytokines, and chemokines, have beneficial effects on injured tissues [27, 28] by promoting angiogenesis and inhibiting fibrosis, apoptosis and inflammation [29, 30]. This hypothesis is supported by preclinical studies showing that many cell types respond to paracrine signaling from MSCs, causing the modulation of a large number of cellular responses, such as survival, proliferation, migration, and gene expression [30]. Various animal models have also demonstrated that MSCs accelerate and promote new bone formation. Factors secreted by MSCs include trophic and immunomodulatory factors [31]; insulin-like
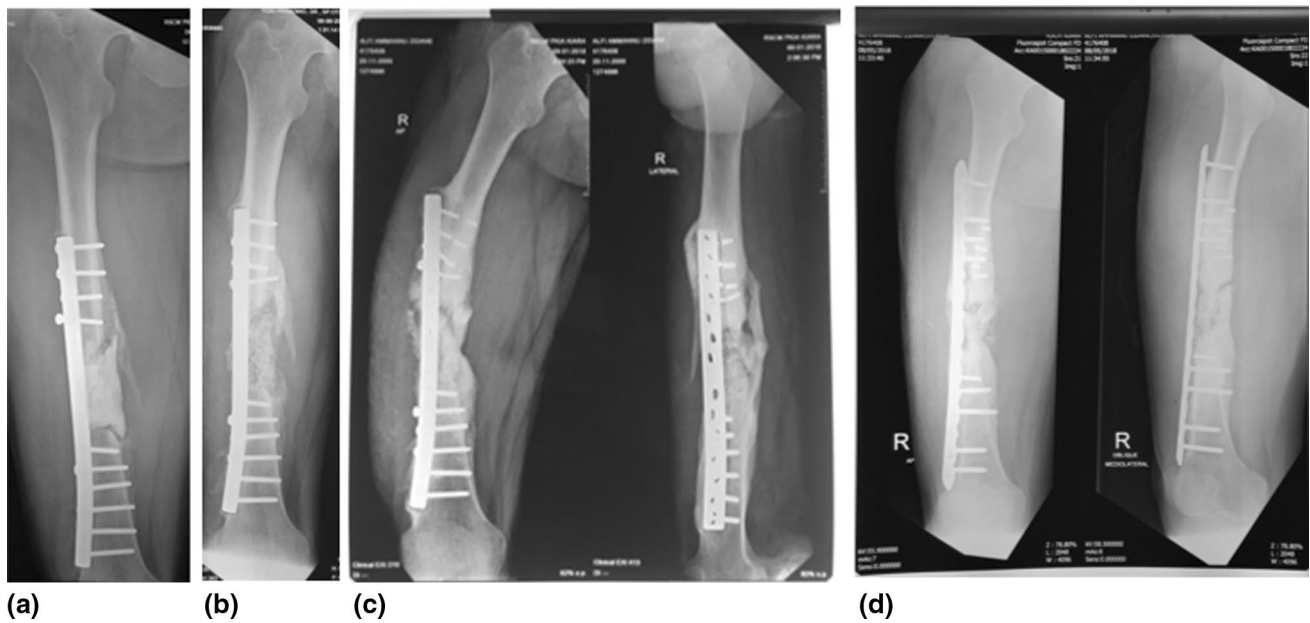
Fig. 2 Femoral radiography of Subject 2a. **a** Preoperative radiography. **b** Postoperative radiography. **c** 6-month postoperative radiography. **d** 12-month postoperative radiography
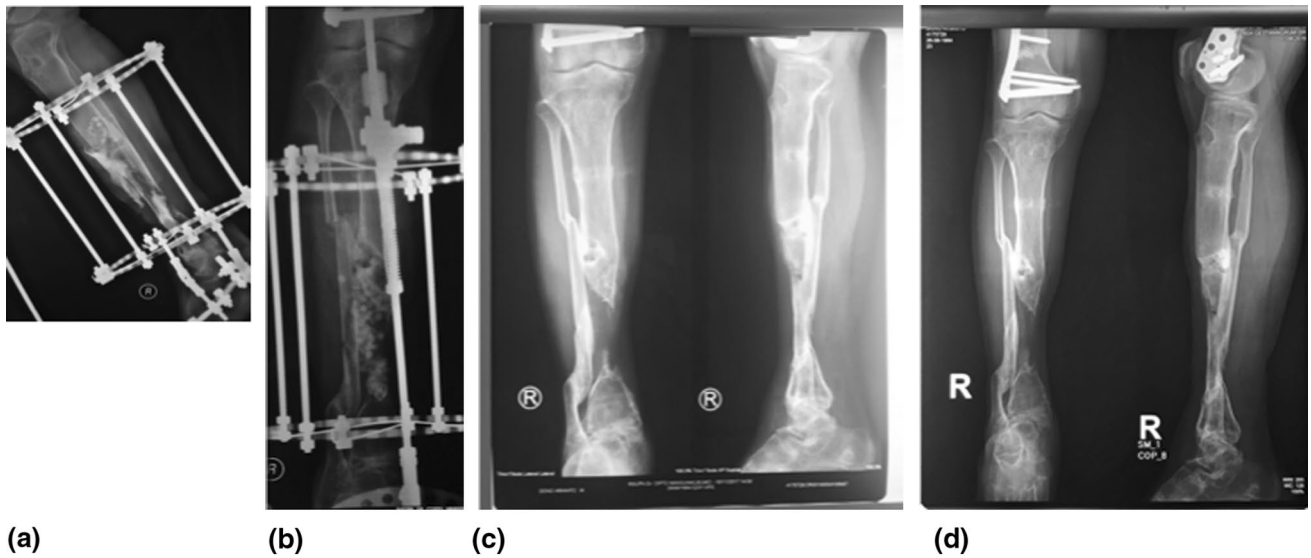

Fig. 3 Tibial radiography of Subject 2b. **a** Preoperative tibial radiography. **b** Postoperative tibial radiography. **c** 6-month postoperative tibial radiography. **d** 12-month postoperative tibial radiography

growth factor-1 (IGF-1) induces osteoblast proliferation and differentiation [32], and vascular endothelial growth factor (VEGF) promotes angiogenesis. VEGF also contributes to osteogenesis by enhancing the survival and differentiation of endothelial cells [33]. TGF-β1 stimulates migration of osteoprogenitor cells, and it also regulates cell proliferation and differentiation. Moreover, it enhances extracellular matrix (ECM) production [34]. All of these factors secreted by MSCs contribute to the bone formation process (Fig. 6).

Previously, MSCs have been found to allow bone regeneration in multiple cases. As far as we are concerned, there is no study regarding the use of UC-MSCs in humans with CSBDs. However, there are several studies that investigate the osteogenic potential of hUC-MSCs. In an in vitro study, Baba et al. found that hUC-MSCs differentiated into osteoblasts and adipose cells, thereby verifying their osteoblastic and adipogenic-differentiation potential [35]. rhBMP-2 was also used in the present study as an osteoinductive factor at the time of differentiation induction. Hydroxyapatite used as the scaffold had already been shown to be a useful scaffold for osteogenesis [35]. In this present study, it was found that the use of hUC-MSCs could probably provide satisfactory outcome for those with CSBD. In cases of CSBD, the direct effect of exogenous hUC-MSC is more prominent in high growth factor-contained induced membrane environment, and MSCs proliferate and differentiate into osteogenic cells (Fig. 7).

Three subjects showed complications of the treatment. One subject (Subject 2) experienced wound dehiscence, surgical site infection, and exposure of the plate. The subcutaneous part of the tibia is susceptible for these complications. However, after the plate had been removed, the infection was solved. Another subject (Subject 4) who developed complications resulted in successful union at the fibular-tibia site, but non-union in the tibial defect site. This was a result of poor soft tissue condition in tibial defect site. Angiogenesis could not occur in poor and shrinkage soft-tissue environment.
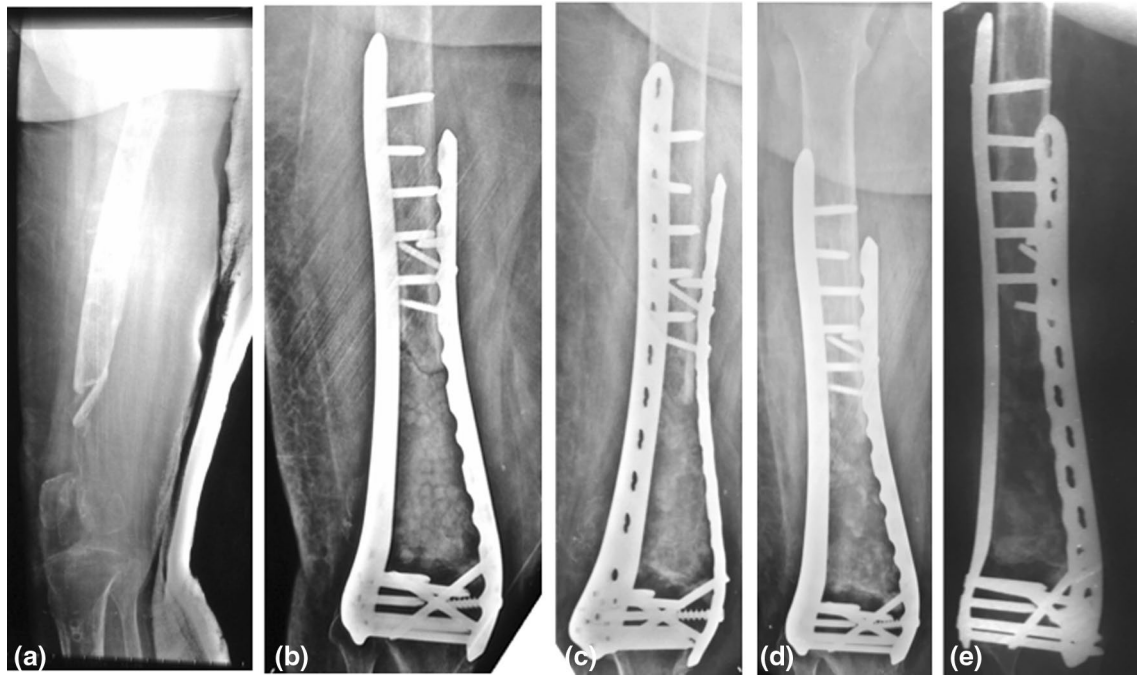
Fig. 4 Tibial radiography of Subject 3. **a** Preoperative radiography. **b** Postoperative radiography. **c** 6-month postoperative radiography. **d** 12-month postoperative radiography

Fig. 5 Femoral radiography of Subject 4. **a** Postoperative radiography. **b** One month postoperative radiography. **c** 3-month postoperative radiography. **d** 6-month postoperative radiography. **e** 12-month postoperative radiography

Therefore, hUC-MSC in favorable soft-tissue environment (e.g., free from infection) is a potential new treatment to treat CSBD (Fig. 8).

The present study highlights that hUC-MSCs could be safely used for treating CSBDs. While this initial study is limited by small number of subjects and the lack of a

Fig. 6 Femoral radiography of Subject 5. **a** Femoral radiography before undergoing Masquelet. **b** Post mesenchymal stem cells implantation femoral radiography. **c** 6-month postoperative femoral radiog-raphy. **d** 12-month postoperative femoral radiography. **e** 15-month postoperative femoral radiography

Fig. 7 Femoral radiography of Subject 6 **a** Preoperative femoral radiography. **b** Postoperative femoral radiography. **c** 6-month postoperative femoral radiography. **d** 12-month postoperative femoral radiography
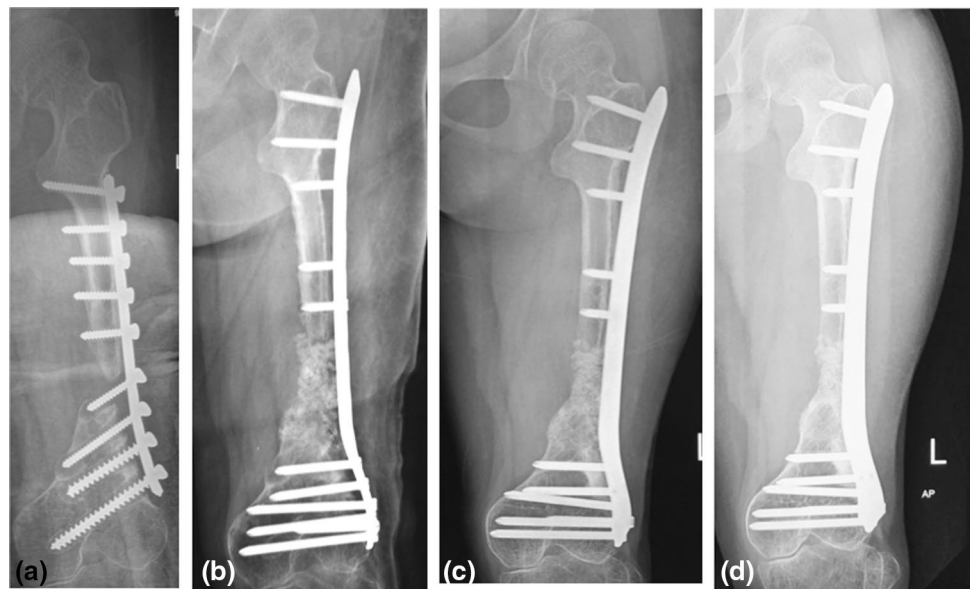
control group, it is our hope that further research regarding the use of hUC-MSCs can be conducted with larger patient cohorts to thoroughly assess the efficacy and safety of the therapeutic use of hUC-MSCs in treating CSBDs.

Conclusion

Umbilical cord-derived mesenchymal stem cells are a potential new treatment for critical size bone defects. This study found significant improvement in terms of LEFS and

Fig. 8 Femoral radiography of Subject 7. **a** Preoperative femoral radiography. **b** Postoperative femoral radiography. **c** 3-month postoperative femoral radiography. **d** 6-month postoperative femoral radiography. **e** 12-month postoperative femoral radiography. **f** 30-month postoperative femoral radiography

VAS of patients diagnosed with lower extremity CSBD. Further studies with larger samples versus controls are required to investigate the safety and efficacy of umbilical cord-derived mesenchymal stem cells for treating critical size bone defects.

Compliance with ethical standards

Conflict of interest Ismail Hadisoebroto Dilogo, Dina Rahmatika, Jeanne Adiwinata Pawitan, Isabella Kurnia Liem, Tri Kurniawati, and Fajar Mujadid declare that they have no conflict of interest.

Ethical approval Ethical approval was received from the Health Research Ethics Committee of Faculty of Medicine, Universitas Indo-nesia—Cipto Mangunkusumo Hospital, Jakarta, Indonesia. The Reference Number was 165/H2.F1/ETIK/2014.

Registration of research studies Clinical Trial Registry Number: NCT 0172 5698.

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