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Stem Cells for Reutilization in Bone Regeneration

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ABSTRACT

Bone is one of the most transplanted tissues. While most bone defects heal spontaneously, critical size defects caused by major trauma/malignant tumor and osteonecrosis of femoral head in young adults pose a great challenge in treatment. While the golden standard in treating bone defects is autologous bone grafting, available bone for grafting is quite limited in an individual. To solve the dilemma, stem cell therapy has been tried as a new modality of treatment in lesions not amenable to autologous bone grafting. While successful results were reported from individual studies, the stem cell therapy is still not an established treatment modality for bone regeneration and needs further assessment. Our focus herein is to introduce stem cell sources that have been investigated so far and review the current status of stem cell reutilization for bone regeneration as well as suggesting future perspectives. J. Cell. Biochem. 116: 487–493, 2015.

KEY WORDS: BONE REGENERATION; STEM CELLS; BONE DEFECTS; OSTEONECROSIS OF FEMORAL HEAD; REUTILIZATION

Bone is one of the most transplanted tissues, more than 2.2 million bone graft procedures being performed every year in the world [Giannoudis et al., 2005]. Bone is unique in that a complete regeneration occurs after injury, rather than healing with a scar formation as is the rule with other tissues. While most bone defects heal spontaneously, critical size defects caused by major trauma or resection of malignant tumor pose a great challenge in treatment. The golden standard in treating bone defects is autologous bone grafting, which provides osteoconduction, osteoinduction as well as osteogenesis. However, autologous bone available for grafting is quite limited in an individual. To treat a large bone defect, a considerable portion of bone should be grafted from other parts of body, creating inevitable morbidity in the donor site. Allograft from cadaver provides more abundant source for bone tissue. However, this method is fraught with the risk of infection and disease transmission, as well as inability to incorporate into host bone if used in bulk form. Bone substitute materials available for surgical treatments have been developed for decades. However, most of these acellular materials are not useful for complex bone reconstructions because they provide only osteoconduction. So, more often than not, they fail to provide a desired clinical outcome.

The osteonecrosis of femoral head (ONFH) is a peculiar disease which occurs in young population, leading to premature total hip arthroplasty (THA). The results of THA in young patients have been improved with recent advancements in biomaterial engineering. Still, replacing a patient's joint to an artificial one when he or she is in twenties or thirties is not a generally acceptable idea. While several treatment methods have been attempted to preserve femoral head in ONFH, most of these procedures have not met with reasonable clinical success so far. A treatment modality that induces the regeneration of bone with minimal surgical intervention is desired in those young patients.

STEM CELLS SOURCES FOR BONE REGENERATION

Stem cells, due to their capacity for proliferation and differentiation into several lineages, have been considered as the prime cell sources for bone regeneration. Stem cell-based approach for bone repair largely emulates autologous bone grafting, which provides osteogenic cells as well as key osteogenic and angiogenic growth factors and templates to recruit host cells which actively lay down bone matrix and vascularize the bone construct [Nukavarapu et al., 2011; Amini et al., 2012].

Thus far, investigated stem cell sources comprise adult stem cells including mesenchymal stem cells (MSCs) and pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Adult MSCs are isolated from bone marrow [Pittenger et al., 1999], skeletal muscle [Alessandri et al., 2004], adipose tissue [Barry and Murphy, 2004], synovial membrane, [De Bari et al., 2001],

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and trabecular bone [Marrony et al., 2003]. MSCs in the bone marrow act as reserve forces for bone repair and regeneration during life period, and therefore have been extensively studied for bone regeneration [Marolt et al., 2010]. Adult stem cells from other adult tissues, particularly adipose stem cells (ASCs), have been also investigated for osteogenesis and repair of skeletal defects in vivo due to their easy accessibility and abundance [Im et al., 2005].

On the other hand, MSCs have disadvantages of limited availability and proliferation, a decrease in regenerative properties with extended expansion [Both et al., 2011] and increasing age of individual [Mareschi et al., 2006; Fan et al., 2010]. These factors limit the use of autologous MSCs in older population who represent a major portion of patients in need of bone replacement [Cancedda et al., 2007]. Accordingly, pluripotent stem cells (PSCs) including embryonic stem cells (ESCs) and induced PSCs (iPSCs) have been investigated as an alternative cell source because they possess an unlimited growth potential that would solve the problems coming from limited proliferation of adult stem cells.

EMBRYONIC STEM CELLS (ESCS)

Since first established more than 30 years ago, ESCs have generated great excitement due to their unlimited proliferation potential that is greatly useful for regenerative medicine. ESCs are usually obtained from extra embryos during in vitro fertilization techniques [Hoffman and Carpenter, 2005]. Successful differentiation of hESCs toward the osteogenic lineage using different methods has been reported both in vitro and in vivo. Osteogenic differentiation of ESCs may be achieved with either first forming or not forming embryoid bodies (EBs). In the first method, EBs are dissociated into single cells, replated, and then administrated with osteogenic supplements [Buttery et al., 2001; Kawaguchi et al., 2005]. In the second method, ESCs undergo direct differentiation into the osteogenic lineage under osteogenic medium, which may provide a more simple and efficient, strategy to obtain bone forming cell [Karp et al., 2006; Hwang et al., 2008].

Despite their enormous potential, concerns about ESCs must be addressed prior to their potential application for bone regeneration. The danger of teratoma formation, elaborate culture conditions including various growth factors, feeder cell layers, specific media and/or coated culture plates, and undetermined immunogenic properties pose a great challenge in using ESCs for regenerative medicine. In addition, the stability of the donor ESCs should be assessed as prolonged culture of undifferentiated ESCs may result in spontaneous development of abnormal karyotypes [Richards et al., 2003].

INDUCED PLURIPOTENT STEM CELLS

Generation of iPSCs by nuclear reprogramming of adult somatic cells allows the preparation of an unlimited number of patient-specific cells for tissue repair. iPSCs have characteristics similar to those of human ESCs, regarding not only morphology, gene expression, surface antigens but also in vitro differentiation potential and pluripotency [Takahashi and Yamanaka, 2006]. While autologous hiPSCs, unlike ESCs, are free from the concern of immune reaction, they are not free from the problems associated with elaborate culture and teratoma formation. In addition, the inherent epigenetic memory of the starting donor cell may influence the differentiation potential and in vivo characteristics of tissues derived from iPSCs [Polo et al., 2010]. Furthermore, possible tumorigenesis due to integrated oncogenes requires special attention and investigation. For nonlethal condition, such as bone defects, it is mandatory to develop non-viral induction methods to produce clinically safe iPSCs. From the author's preliminary study on the osteogenic potential of hiPSCS, iPSCs showed comparable in vivo bone formation in immunosuppressed rats while demonstrating delayed in vitro osteogenic differentiation when compared with MSCs [Ko et al., 2014] (Fig. 1).

ADULT STEM CELLS

MSCs have been isolated from a number of adult sources using a relatively simple protocol that primarily relies on their adhesion to plastic surface in culture [Caplan, 1991]. Cultured MSCs exhibit a low immunogenic phenotype including absence of MHC Class II antigen. MSCs have been shown to suppress the proliferation of T cells and production of cytokine, and inhibit the function of B cells, dendritic cells, and the natural killer cells. These characteristics greatly enhance the therapeutic advantage of MSCs [Law and Chaudhuri, 2013].

In addition to adult tissues, MSCs have recently been derived from ESCs and iPSCs. These ESC- and iPSC-derived MSCs have the same in vitro and in vivo multi-potent characteristics as MSCs derived from other adult sources. However, unlike MSCs derived from adult sources, iPSC-derived MSCs show a lower rate of senescence when expanded in culture due to higher telomerase activity [Yu et al., 2007]. As mentioned previously, MSCs of embryonic and iPSC origin must be further tested for safety before they are considered for clinical application.

MSCs can be applied to the bone defect along with biomaterials in order to accelerate bone formation. MSCs provide osteogenic cells while biomaterials impart enhanced osteoconduction and osteoinduction by releasing osteogenic growth factors and stimulating the migration and differentiation of host osteoprogenitor cells. Differentiating MSCs into the osteogenic cells before implantation can further accelerate the repair of bone defect and osteointegration of MSC-biomaterial construct in vivo [Mauney et al., 2005].

There are several factors that significantly limit the actual amount and the quality of MSCs available for clinical application. First, MSCs reach senescence-associated growth arrest after a maximum of 24–40 population doublings. Also, with increasing donor age and systemic disease, in vitro osteogenic differentiation potential and in vivo bone formation significantly decreases [Arvidson et al., 2011; Kagami et al., 2011]. In addition, long-term culture may increase the possibility of abnormal karyotype development and malignant cell transformation by forced selection under artificial conditions [Rubio et al., 2005; Rosland et al., 2009; Amini et al., 2012].

DELIVERY OF STEM CELLS TO PROMOTE BONE HEALING

ORTHOTOPIC APPLICATIONS

Most common method for administering stem cells to repair bone defect is direct implantation with or without scaffolds. A large number

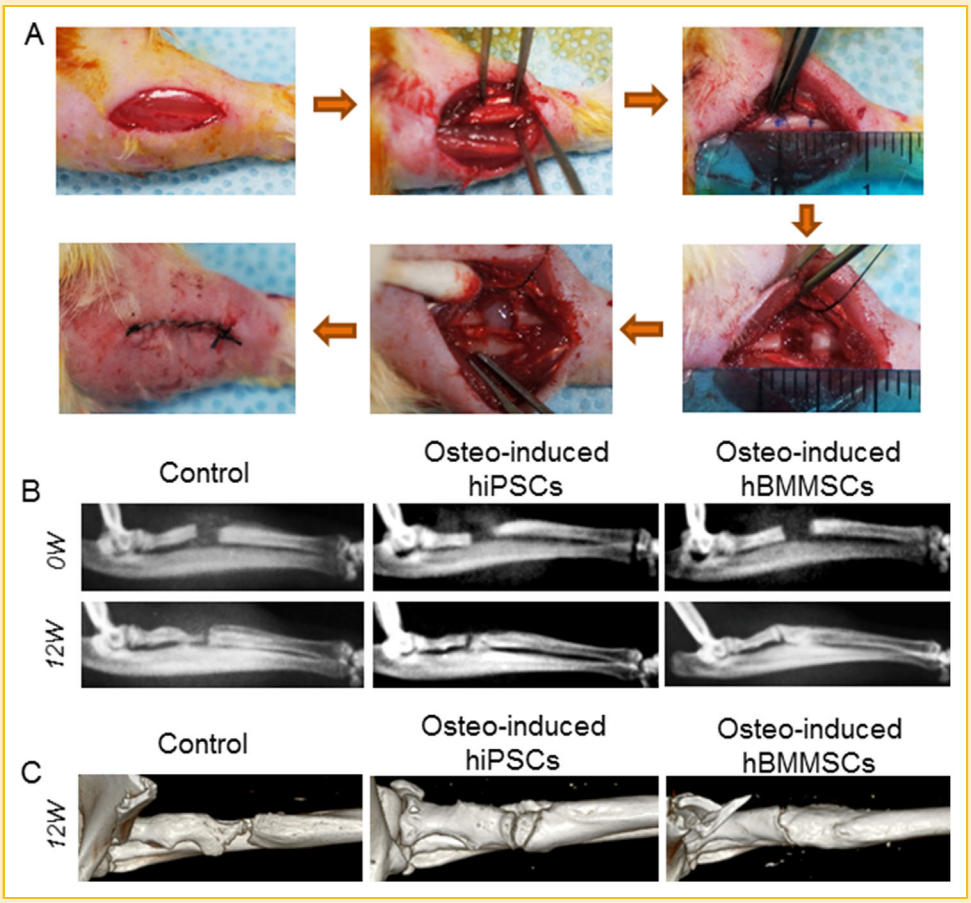

Fig. 1. Healing of critical-size long bone segmental defect by osteo-induced hiPSCs and osteo-induced human bone marrow MSCs (hBMMSCs). (A) Creation of segmental defects from radial shaft of rats. (B) Findings from plain radiographs immediately and 12 weeks after implantation. (C) CT findings after 12 weeks of implantation (Reproduced with permission from Ko et al. Stem Cell Dev. 2014;23(15):1788–97).

of preclinical studies and a small number of human trials showed the efficacy of direct orthotopic delivery of expanded MSCs into large segmental defects. The nature of the scaffold influences the performance of the MSC implantation. To date, scaffolds that contain bioceramics [usually hydroxyapatite/β-tricalcium phosphate (HA/β-TCP)] as part of their composition are the most reliable carrier for bone formation when seeded with MSCs [Oh et al., 2006]. As bioceramics demonstrate slow in vivo resorption, scaffolds composed of polymers, such as poly(lactic-co-glycolic acid) and poly(e-caprolactone), with and without calcium phosphate components or further functionalization, are also available for more rapid resorption [Rezwan et al., 2006; Kretlow and Mikos, 2007].

Instead of solid forms, the use of injectable scaffolds that carry stem cells into the bone defect and support differentiation can avoid the need for open surgery for stem cell implantation in appropriate cases [Mankani et al., 2008]. This concept can be applied to the treatment of ONFH, benign cystic bone lesion and nonunion.

SYSTEMIC APPLICATION

MSCs can be also delivered through the systemic circulation. MSCs are known to home directly to sites of bone injury when administered systemically although it is not certain whether they directly contribute to healing by becoming bone-forming cells [Robey, 2011]. In uninjured animals, systemically administered MSCs (either autologous or allogeneic) are predominantly trapped in the lungs. Although systemic infusion of autologous or allogeneic MSCs does not lead to a broad distribution of cells that persist for long periods, beneficial effects comes from copious amounts of a large repertoire of growth factors and cytokines that are secreted from MSCs. These factors may indirectly promote bone healing by recruiting other cell-types and increasing vascularization. The immunomodulatory properties of MSCs would also play a role in bone formation by reducing inflammation that suppresses endogenous bone regeneration [Robey, 2011].

CLINICAL APPLICATION OF STEM CELLS TO HEAL BONE DEFECT

Haynesworth et al. first implanted human bone marrow MSCs with ceramic scaffolds to generate ectopic bone in vivo in immunodeficient mice [Haynesworth et al., 1992]. This study provided proof-of principle on the feasibility of using hMSCs in bone regeneration. The

repair of critical-sized bone defects using stem cells has been reported by a number of animal studies thereafter [Hanada et al., 1997; Bruder et al., 1998; Kon et al., 2000; Arinzeh et al., 2003; Kruyt et al., 2003, 2004; Bensaid et al., 2005; Kruyt et al., 2006; Mankani et al., 2006; Mastrogiacomo et al., 2006; Siddappa et al., 2008]. Most importantly, several clinical studies have been conducted to assess the safety and efficacy of this approach in humans. Nevertheless, stem cell implantation for bone regeneration is still not a routine, established clinical practice at this time.

Most of human studies described in literature for stem cell-based bone regeneration are cohort outcome studies or case reports due to practical and ethical issues involved in conducting a randomized controlled trial. Absence of controls is a major drawback of cohort studies. However, these preliminary studies at least provide some clues to the safety and potential therapeutic effects of the treatment. However, randomized controlled trials should be performed in the future before stem cell therapy enters the realm of routine clinical practice although these studies are costly, time consuming, and need elaboration [Chatterjea et al., 2010].

There is a small number of human clinical studies or case reports that have been published in literature, using autologous, culture expanded, nongenetically modified human MSCs for bone regeneration. The ethical approval for conducting these studies has been provided by local ethics committees of each author's institution.

The first clinical case series described the preliminary results of three patients who had various segmental long bone defects and were treated with implantation of autologous MSCs [Quarto et al., 2001]. Macroporous 100% hydroxyapatite (HA) scaffolds that were custom made to fit the shape and size of the defect were loaded with ex vivo expanded hMSCs isolated from their own bone marrow and implanted into the defects. After follow-up of 6–7 years, the implants displayed good osseointegration with no further complications. Although no controls were included in this study and the assessment of results was based only on radiological evaluation, the study showed that the procedure was safe to perform. In another attempt, one patient with a comminuted fracture of femur was treated using a combination of autologous cancellous bone and stem cell-seeded porous calcium-triphosphate granules in the ratio of 1:2 [Stres et al., 2007]. Most of other clinical studies were all performed in dental areas to reconstruct maxillary or mandibular defect using various scaffolding materials including fibrin glue, β-TCP or HA granules, and platelet rich plasma. [Hibi et al., 2006; Meijer et al., 2008; Shayesteh et al., 2008; Mesimaki et al., 2009; Correia et al., 2011].

The clinical studies conducted so far have demonstrated that it is safe to use autologous hMSCs for bone regeneration. None of the reports mention adverse effects, such as inflammation or excessive tissue growth, although several in vitro studies suggest that extensively cultured MSCs (4–5 months) can develop genomic instability, an indicator of malignant transformation [Rubio et al., 2005; Rosland et al., 2009]. The relatively short period (6–8 weeks) needed to obtain sufficient cell numbers in most clinical applications, may account for the lack of reported malignancy in the clinical studies performed so far. However, for the extended application of cultured stem cells, chromosomal analysis is necessary to ensure safety for the recipient patient. In addition, most of the published clinical studies have a short follow-up period. Longer follow-up periods would be necessary to obtain data on the definite safety of stem cell implantation for bone regeneration.

It should be also taken into account that the expansion of hMSCs in culture has unfavorable effects on their differentiation potential [Banfi et al., 2000; Alves et al., 2009]. To avoid unphysiological expansion of MSCs, other sources of adult stem cells, such as umbilical cord, human placenta, amniotic fluid may be used to provide osteogenic cells. In order to enhance the expression of osteogenic genes while maintaining acceptable costs and safety profile, MSCs can be primed using growth factors such as BMPs [Cowan et al., 2005; Garrison et al., 2007] or vitamin D [Song et al., 2011] to enhance the bone-forming capacity.

The fact that MSCs lack certain surface markers responsible for the host T-cell response opens up possibilities for using such allogeneic implantation of stem cells with proven bone-forming potential [Tse et al., 2003; Tasso and Pennesi, 2009]. A standardized off-the-shelf product may be routinely applied to the clinic. However, data from clinical reports using allogeneic MSCs for bone regeneration would be needed to consider them as a viable option for treating bone defects.

APPLICATION OF STEM CELLS FOR OSTEONECROSIS

While the etiology of ONFH has been focused on disturbances in thrombosis or coagulation, recent data show that decreased osteogenic potential in stem cells of patients also plays a role in the pathogenesis of ONFH. Decrease in the number of MSCs was found in the proximal femur outside of the area of osteonecrotic lesion, reflecting a global reduction in MSCs in the site [Hernigou and Beaujean, 1997]. Furthermore, evidences were found that MSCs in the femoral head of ONFH patients are not as active as those in normal femoral heads [Gangji et al., 2003; Suh et al., 2005]. These findings provide a rationale for a cell therapy in the disease.

The idea of treating ONFH by cell therapy started from the attempt of Hernigou et al. who added the bone marrow aspirate concentrates (BMAC) to core decompression [Hernigou and Beaujean, 2002]. Although there is a paucity of randomized prospective trial [Gangji et al., 2011], series of publication reported the usefulness of the implantation of the concentrated bone marrow aspirate to treat ONFH [Hernigou et al., 2009].

Questions need to be answered in order to establish a protocol for clinical applications are: (1) which cells will be effective? (2) is carrier material necessary? and (3) which route of administration is to be used? Most of published series used direct injection of BMAC. While this procedure obviates the need for cell isolation and subsequent culture, and thereby reduce the cost, BMAC is a mixture of various mononuclear cell and only a small fraction (0.01%) comprise MSCs [Jones et al., 2006]. Furthermore, aspiration from several sites is necessary to obtain enough amount of BMAC for aspirate. The amount of stem cells in the final preparation varies from a patient to another, and thus cannot be standardized. These difficulties prevent the use of BMAC from becoming a universal procedure.

Ex vivo expanded autologous MSCs have been used in treating ONFH and met with similar success as BMAC [Kawate et al., 2006; Gangji and Hauzeur, 2009]. When patients are young, and ex vivo

expansion of bone marrow MSCs can provide enough cells for implantation. Allogeneic MSCs can be potentially considered although there are scanty reports on the application of allogeneic MSCs to treat ONFH. Hernigou et al [Hernigou et al., 1997] used allogeneic stem cells by intravenous route in a patient who had osteonecrosis of the humeral head secondary to sickle-cell disease, leading to a favorable outcome and total repair of the osteonecrosis after a follow-up of 4 years. The use of allogenic instead of autologous MSCs for the treatment of ONFH appears attractive because of logistic and economic advantages given that these cells might be available as an "off the shelf" product although clinical data on the efficacy and safety are necessary to consider the application of allogeneic MSCs for ONFH. Successful result with intra-arterial injection via of autologous bone marrow enriched with MSCs was also reported [Mao et al., 2013]. The working mechanism and safety issues of this approach should be further investigated and corroborated by other studies.

While earlier studies did not utilized carrier material, scaffolds in the form of hydrogel or solid forms help to retain the cells on the lesion site and to promote osteoconduction or osteoinduction. The use of fibrin glue and β-TCP ceramic chips have been reported along with BMAC or expanded MSCs [Kawate et al., 2006; Lim et al., 2013].

CONCLUDING REMARKS

Cell therapy has been considered and tried as a new modality of treatment in a large skeletal defect not amenable to autologous bone grafting and ONFH in young patients in last two decades. While successful results were reported from individual studies, the stem cell therapy is not an established treatment modality for bone regeneration and needs further investigation and assessment. For successful clinical application to regenerate bone, several factors should be taken into consideration including isolation and expansion efficiency, expression and stability of osteogenic markers, and long-term safety including immune reaction and tumorigenicity. MSCs are currently leading cell sources for bone regeneration. However, an ex vivo expansion method that maintains the biological properties of the cells is essential for clinical translation. Advancements in scaffolds and carriers are also necessary for better results. PSC technology offers a possibility for creating large numbers of cells while it will take a time before safety issues are resolved. To be used for clinical application, the protocols to induce progenitors of the mesenchymal lineages from PSCs should be established and the safety issue including immunogenicity and tumorigenecity should be solved.

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