Histol Histopathol (2014) 29: 691-697
DOI: 10.14670/HH-29.691

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Current trends in stem cell therapy for improvement of bone quality

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Summary. As the average lifespan of humans continues to increase, improvement in the quality of life for elderly people is important. Among the most severe problems during aging are bone loss-associated diseases such as poor fracture healing and osteoporosis. Therapy-induced bone loss such as bisphosphonate-associated osteonecrosis of the jaw also increases in incidence with age. Most of the current treatment strategies are focused on antiresorptive and bone formation pharmacological agents, but it is hard to obtain appropriate bone augmentation and there are concerns regarding their long-term safety without side effects. Therefore, a novel method for improvement of bone quality is required, and stem cells are of great interest as potential therapeutic tools for diseases that remain without clinically effective therapies. In this review, we describe the concept of stem cell-based therapy and evaluate the current progress of cell therapy for the improvement of bone quality. In addition, we report and discuss a new clinical strategy in which improved bone quality was obtained by applying bone-marrow derived MSCs with platelet rich plasma in clinical therapy.

Key words: Improvement of bone quality, Regenerative medicine, Mesenchymal stem cells (MSCs), Minimal invasiveness, Stem cell therapy

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Introduction

Abnormalities in the quantity and quality of bone tissue lead to impaired skeletal strength, bone loss, and increased risk of fractures (Lane et al., 1997). Approximately 52 million people in the United States are affected by low bone density or osteoporosis (John, 2007). Most of the current treatment strategies are focused on antiresorptive and bone formation pharmacological agents, including bisphosphonates, drug altering estrogen receptor activation, calcitonin, and anabolic therapies. However, several of these drugs prevent progression of disease but do not stimulate bone growth (Valverde, 2008). Moreover, there have been ongoing concerns regarding their long-term safety. For example, long-term use of hormone replacement therapy can increase the risk of breast cancer, stroke and embolism, and bisphosphonate treatment may lead to severe suppression of bone turnover and osteonecrosis of the jaw (Odvina et al., 2005; Valverde, 2008). Therefore, the development of new therapeutic modalities will be necessary.

Recently, tissue engineering and regenerative medicine has received much attention and is expected to be a promising approach for various diseases. Stem cells will be critical for the development of new treatment options for tissue regeneration. This article reviews the current trends in stem cell therapy for bone loss-associated diseases. Furthermore, it will describe a novel clinical strategy for improvement of bone quality.

Concepts of tissue engineering and regenerative medicine for bone regeneration

The goal of tissue engineering and regenerative medicine has been to restore and reconstruct damaged or lost tissue and to improve function. The field attempts to regenerate tissues or organs by using three elements: stem cells, scaffolds and growth factors (Bianco and Robey, 2001). Stem cells are a critical factor in regeneration of tissues or organs, and they have received a great deal of interest as potential therapeutic tools for various chronic debilitating diseases for which there are no clinically effective therapies. These cells are able to differentiate into multiple cell types to promote regeneration, and the most convenient means of cell delivery has been direct injection or infusion of a suspension of cells into the disease site (Mooney and Vandenburgh, 2008).

Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have revolutionized cell therapies and regenerative medicine because of their full pluripotent differentiation potential. Pluripotent stem cells will be a powerful strategy to treat diseases, not only as cell-based therapies, but also as disease models including theories of disease mechanisms and novel inspiration for therapeutic drugs (Cherry and Daley, 2013). However, the ethical issues involved in destroying human embryos and the immune reactions that occur after transplantation are two major stumbling blocks impeding the clinical application of ES cells. Several issues, including safety concerns such as the risk of cancer, and the maintenance and differentiation of stem cells still remain unclear with regard to using ES or iPS cells for clinical application.

On the other hand, mesenchymal stem cells (MSCs) are multi-potent stem cells that were originally introduced in the 1960s, which had the ability to differentiate into osteoblasts (Friedenstein et al., 1968). In recent years, MSCs-based therapeutic applications have received intensive attention not only because of the stem cells' self-renewal and trans-differentiation potential, but also due to their immunosuppressive and immunomodulatory effects on lymphocytes and other proinflammatory cells (Di Nicola et al., 2002; Ren et al., 2009). These characteristics have made MSCs a promising candidate for cell therapy in many refractory diseases, including bone and joint diseases such as osteoarthritis, rheumatoid arthritis, osteoporosis, osteonecrosis of the femoral head, and osteogenesis imperfecta (Liu et al., 2014).

Bone regeneration by tissue engineering using MSCs extracted from various tissues, including bone marrow, adipose, dermis, neural tissue, periodontal ligament, and dental pulp (Pittenger et al., 1999; Kern et al., 2006; Yamada et al., 2010), is favorable for elderly patients because it is relatively noninvasive. These cells can be obtained under local anesthesia and propagated *ex vivo* easily. The concept of bone regeneration using MSCs is direct delivery of osteogenic cells into the defect site with the aid of scaffolds in combination with growth factors. Quarto et al (2001) reported the use of MSCs-based tissue-engineering approach to treat three patients with large bone defects in orthopedic surgery and which resulted in substantial improvement in their ability to repair large defects in long bones (Quarto et al., 2001). Ohgushi et al. (2005) applied bone marrow-derived mesenchymal stem cells (BMMSCs) to three patients suffering from osteoarthritis (Ohgushi et al., 2005). They applied BMMSCs to ceramic ankle prosthesis and cultured them to form an osteoblasts/bone matrix on the prosthesis, and high clinical scores with stable host bone–prosthesis interface were obtained. We have previously reported clinical studies with a combination of BMMSCs and Platelet Rich Plasma (PRP) for bone augmentation of alveolar bone (Yamada et al., 2008, 2013). The patients exhibited significantly improved bone volume with no side effects, and no significant bone resorption occurred during long-term follow-up.

Low quality of bones

As society ages, bone loss-associated diseases have an increasing impact on quality of life. These are characterized by fragile bones or low bone mineral density (BMD) with decreased microstructures and low quality of bones, which are easily fractured (Tsolaki et al., 2009). For example, osteoporosis is a common, age-related bone disease characterized by the loss of bone mass, decreased strength (bone quality), and micro-architectural deterioration of bone tissue. It results from an imbalance between the processes of bone formation and bone resorption, leading to fragility and fractures. Approximately 50% of 65-year-old women ultimately experience an osteoporotic fracture during their lives (Liu et al., 2014).

Bone quality is one of the most important aspects in the dental field as well. Dental function is important not only for mastication, including nutritional intake, but also for speech, aesthetics, and psychosocial functions such as satisfaction and social well-being (Trulsson et al., 2012). Endosseous dental implants are a commonly accepted treatment option to restore masticatory functions after tooth loss. The most important factor for successful dental implant treatment is bone quality (Wood and Vermilyea, 2004), but many patients who need treatment with dental implants are elderly, and they are afflicted with health problems and risk factors such as Paget's disease, breast cancer, periodontal disease, or osteoporosis.

This lower bone density and reduced healing capacity might be due to the defects of MSCs with lower proliferation and osteoblast differentiation (Wang et al., 2006). In a previous study, Bruder et al first proposed that systemic administration of culture-expanded autologous MSCs would be a therapeutic option to treat many clinically challenging diseases such as

osteoporosis (Bruder et al., 1994).

Cell therapy for improvement of bone quality

Currently published English language articles that applied cells as a part of the treatment protocol for improvement of bone quality in rat, rabbit, and human were reviewed (Table 1). Because of the limited number of studies, the number of treated cases was not considered as an inclusion criterion. Only five reports, including 4 animal studies and 1 preliminary clinical application, have been published to date. In one animal study, the transplantation of autologous BMMSCs from ovariectomized (OVX) rabbits in femurs showed significant improvement in bone histomorphometrical structure and mechanical strength (Wang et al., 2006). It was also shown that intra-bone injection of BMMSCs into the femur of osteoporotic female rats improves bone mass (Ocarino Nde et al., 2010). Histomorphometric and histological analyses clearly revealed improvements in the BMMSCs treated group as compared to the untreated group, and trabecular bone percentage in the treated group was similar to the femurs from control healthy rats. Hsiao et al used segregated BMMSCs from haematopoietic cell contaminants by a single-step plastic-adherent method for intravenous transplantation into osteoporotic mice and showed rescue ability from osteoporosis symptoms (Hsiao et al., 2010). Cho et al studied the effects of intravenous transplantation of BMMSCs overexpressing receptor activator of nuclear factor- kappa B (RANK)-Fc and CXC chemokine receptor-4 (CXCR4) using retrovirus on OVX-induced bone loss in mice (Cho et al., 2009). They found that MSCs overexpressing RANK-Fc effectively prevented OVX-induced bone loss and co-overexpression of CXCR4 resulted in greater protection. A final case study reported cell therapy using allogeneic umbilical cord blood mononuclear cells to idiopathic osteoporosis patients (Li et al., 2012). They subcutaneously injected the cells in the left arm of every patient and demonstrated a beneficial effect on bone density. As described above, in animal studies it has been reported that bone quality can be improved by BMMSCs, and the effects of the cells, which remain localized long term, can contribute to the improvement of bone quality. However, until recently no human clinical study using MSCs for low bone density disease was reported.

A novel strategy for improvement of bone quality using MSCs

Our previous clinical studies showed effective outcome of MSCs-based tissue-engineering approach for bone regeneration (Yamada et al., 2008, 2013). Therefore, we applied BMMSCs to a 55-year-old female patient to improve bone quality. The concept of this

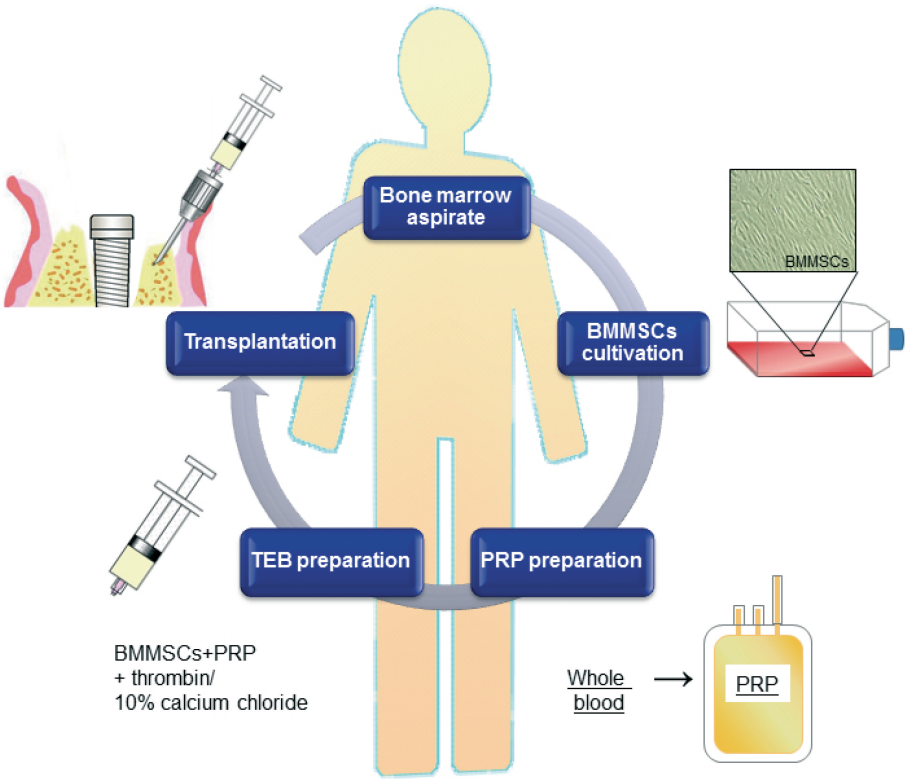

Fig. 1. Treatment protocol schema.

strategy is shown in Fig. 1. The patient had lost first and second molar teeth in the mandible. The healing was insufficient and the bone quality was low. It was confirmed clinically and radiographically (Fig. 2), and an improvement of bone quality was needed for implant placement. The patient was generally in good health and free from any disease that may have influenced the treatment outcome (e.g., diabetes, immunosuppressive chemotherapy, rheumatoid arthritis). The patient was informed about the procedures, including the surgery, graft material, implants and uncertainties of using a new bone-regenerative method, and the university ethics committee approved the research protocol. The patient agreed and chose dental implants with minimally invasive bone regeneration using tissue engineered bone (TEB) composed of BMMSCs and PRP.

Transplantation of Injectable tissue-engineered bone (TEB)

MSCs were isolated from the patient's iliac crest marrow aspirates and PRP was prepared in a 200 ml collection bag containing the anticoagulant citrate under sterilized conditions according to our reported method (Yamada et al., 2008, 2013). The values confirmed the platelet sequestration ability of the process, which showed that the concentration was 257 % above the baseline platelet counts.

Injectable tissue-engineered bone (TEB) was prepared according to our reported method (Yamada et al., 2008, 2013). Briefly, human thrombin in a powder form (5,000 units) was dissolved in 10% calcium chloride. This was aspirated into a sterile syringe and combined with BMMSCs and PRP. After two 13-mm long dental implants were inserted, TEB was injected with a bone biopsy needle (Fig. 2A). The buccal and labial periosteum was extended in the customary manner.

Clinical outcomes

When the usual dental implant second surgery was performed, all inserted implants appeared to be fully integrated. Oral implant bridges were achieved and occlusal function was recovered (Fig. 2A). The patient was followed up with clinical and radiographic examinations. Computed tomography (CT) analysis revealed that the bone density, which is an indicator of bone quality, had increased and the Hounsfield unit (Hu) value (471, 517, 616, 626 Hu at 3, 6, 12, 24 months, respectively) was higher than the pre-operation baseline (149 Hu) and reached native bone level (Fig. 2B,C). The 2-year follow-up examination showed no signs or symptoms of implant failure, and bone quality remained high. Importantly, this improvement was stable over time. Histological observation also indicated good bone formation with a lamellar pattern and well-differentiated marrow cavity (Fig. 2D).

Usefulness of stem cell-based therapy on bone loss-associated disease

Previous studies reported that MSCs were able to migrate, engraft into injured sites, and regulate the repair process by undergoing site-specific differentiation and providing a hospitable microenvironment (Shi et al., 2010; Liu et al., 2014). Previous animal studies suggested that the transplantation of autologous or allogeneic MSCs was able to strengthen bone in osteoporosis animal models (Wang et al., 2006; Hsiao et al., 2010; Ocarino Nde et al., 2010). It was also reported that MSCs were capable of homing to the surface of trabecular bone after directed injection (Ocarino Nde et al., 2010). Therefore, injected BMMSCs might be able to participate in the repair of damaged or diseased bone tissue, especially in cell-poor environments that require a large number of site-specific cells for repair.

The combination of stem cells with biomaterials or growth factors enhances the efficacy of cell therapy in several ways (Sun et al., 2012). We injected the stem cells with PRP, which contains growth factors such as platelet derived growth factor (PDGF), transforming growth factor beta (TGF-beta), and insulin growth factor (IGF). These factors can promote wound healing and

Table. 1. Cell therapy studies to improve bone quality

Authors	Year	Species	Model	Route of Administration	Source	Scaffold	Subject Number
Wang et al.	2006	Rabbit	OVX	Injected into the cancellous space of the femur	BMMSCs	Calcium alginete gels	12 OVX rabbits
Cho et al.	2009	Mouse	OVX	Intravenous	BMMSCs overexpressing RANK-Fc or CXCR4	-	Sham-op + PBS (n=12), RANK-Fc + CXCR4 (n=6), RANK-Fc +GFP (n=6), CXCR4 + RED (n=6), RED + GFP (n=9), or OVX + PBS (n=9)
Ocarino et al.	2010	Rat	OVX	injected into the bone marrow cavity of the femur	BMMSCs	-	control (n=5), osteoporotic rats with injection of PBS (n=5), osteoporotic rats with injection of BMMSCs (n=5)
Hsiao et al.	2010	Mouse	OVX	Intravenous	BMMSCs using single-step plastic-adherent method	-	n=7 mice/group
Li et al.	2012	Human	Idiopathic osteoporosis	Subcutaneously injected in the left arm	Allogeneic cord blood mononuclear cells	-	8 patients

enhance bone regeneration (Marx et al., 1998). PRP activated by thrombin secretes bioactive factors and has an effect on injected cells, which may increase therapeutic efficacy. Moreover, cells fabricate their own natural matrix structure, which provides structural integrity, and in addition, fabricated scaffolds can be

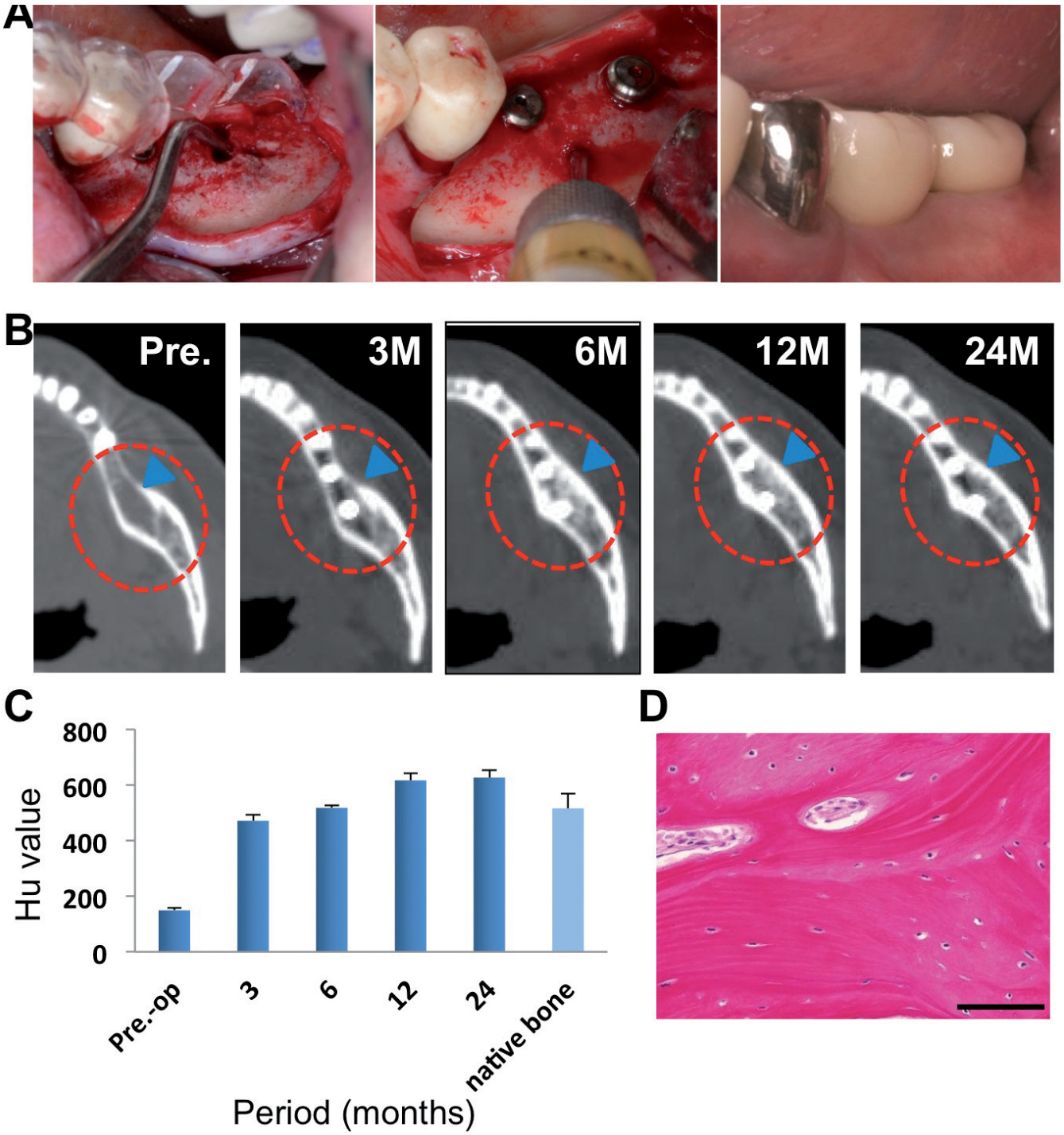

Fig. 2. Clinical application case. **A.** Pre-operation macroscopic view of incomplete healing and crumbly bone tissue (left panel). Macroscopic view on operation. The BMMSCs and PRP were injected to the area (center panel). Prosthesis setting condition (right panel). **B.** CT evaluation demonstrating bone density at pre-operation, 3, 6, 12, and 24 months after injection. The radiopaque area gradually increases. **C.** Change of CT Hu value. **D.** Histological observation of regenerated tissue in the injection area. Good bone formation was observed. Bar: 100 μm.

used to help ensure appropriate differentiation of their progeny (Davies et al., 2010). Such a cell therapy would affect from the specialized microenvironments or niches that normally house stem cell populations within tissues (Mitsiadis et al., 2007). Therefore, the tissue engineering concepts composed of three elements; stem cells, growth factor, and scaffold, may be fundamental to treat bone loss-associated disease.

Conclusion

In conclusion, the application of minimally invasive stem cell therapy-based tissue engineering technology would be useful for improvement of bone quality and provides a long-term, stable outcome. It also would be of benefit to improve bone quality in conditions such as osteoporosis of the jaw. Furthermore, this novel cellular treatment technique will help to increase the rate of osseous integration, reduce healing time and expedite the clinical development of novel therapy options in orthopedics, plastic surgery, dentistry, and oral and maxillofacial surgery.

Acknowledgements. The authors wish to thank members of the Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine and Dr. Koji Yamamoto at the blood transfusion service department of Nagoya University Hospital for their help, encouragement, and contributions to the completion of this study. There is no conflict of interest.

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Accepted December 2, 2013