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ORIGINAL ARTICLE

Unrelated cord blood transplantation vs related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction

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Little information is available regarding whether an unrelated cord blood (UCB) unit or a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the graft-versus-host direction (RD/1AG-MM-GVH) should be selected as an alternative donor for patients without an HLA-matched related/unrelated donor. Therefore, we conducted a retrospective study using national registry data on patients with leukemia or myelodysplastic syndrome who received transplantation using a single UCB ($n = 2288$) unit or an RD/1AG-MM-GVH ($n = 525$). We found that the survival rate in the UCB group was comparable to that in the RD/1AG-MM-GVH group, although the RD/1AG-MM-GVH group with an HLA-B mismatch showed significantly higher overall and non-relapse mortality. Neutrophil and platelet engraftment were significantly faster, whereas the incidence of acute or chronic graft-versus-host disease (GVHD) was significantly higher in the RD/1AG-MM-GVH group. The incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group with *in vivo* T-cell depletion was comparable to that in the UCB group, which translated into a trend toward better overall survival, regardless of the presence of an HLA-B mismatch. In conclusion, UCB and RD/1AG-MM-GVH are comparable for use as an alternative donor, except for RD/1AG-MM-GVH involving an HLA-B mismatch.

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Keywords: cord blood transplantation; related transplantation; HLA mismatch; alternative donor

INTRODUCTION

For patients who lack an HLA-identical sibling, an HLA-matched unrelated donor (MUD) is considered to be the preferred alternative donor in allogeneic hematopoietic cell transplantation (HCT).[1–5] However, it is difficult to find an MUD for patients with rare HLA haplotypes. Furthermore, it takes at least a few months from the start of an unrelated donor search to actually receive a graft. Therefore, there is a large demand for an alternative source to an HLA-identical sibling or MUD, particularly for patients who have a rare haplotype or who need immediate transplantation.

Unrelated cord blood (UCB) has emerged as a promising alternative source for pediatric and adult patients.[6–17] In UCB transplantation, up to two antigen/allele mismatches between a recipient and cord blood unit are acceptable without an increased risk of acute graft-versus-host disease (GVHD). The clinical outcome in UCB transplantation is improving, and is almost comparable to that in HLA 8/8 allele MUD transplantation, although a high risk of graft failure and early treatment-related complications are still major issues.[15–17]

Another alternative source is an HLA-mismatched related donor, particularly when a related donor with a 1-antigen mismatch at the HLA-A, HLA-B, or HLA-DR locus in the graft-versus-host (GVH) direction (RD/1AG-MM-GVH) is available. HCT from an RD/1AG-MM-GVH results in a higher but acceptable incidence of acute GVHD.[18–20] In previous studies, HLA mismatches in the host-versus-graft (HVG) direction were associated with a higher incidence of graft failure and lower overall survival (OS).[18,19,21] However, the risk of graft failure might have been improved by the use of conditioning regimens that strongly suppress the recipient's immune system.[22] Therefore, in current clinical practice in Japan, stem cell transplantation from an RD/1AG-MM-GVH is being performed while accepting multiple antigen mismatches in the HVG direction without specific *ex vivo* stem cell manipulation.[18,19,23] We have recently reported that OS in transplantation from an RD/1AG-MM-GVH involving an HLA-B antigen mismatch was inferior, whereas that from an RD/1AG-MM-GVH involving an HLA-A or -DR antigen mismatch was comparable to that from an 8/8-MUD in standard-risk diseases.[23]

Unlike transplantation from an MUD, transplantation using a UCB unit or an RD/1AG-MM-GVH can be performed immediately when necessary. However, little information is available regarding the priority in selecting these alternative donors. Therefore, we conducted a retrospective study using national registry data on 2813 patients with leukemia or myelodysplastic syndrome (MDS)

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who received transplantation using a single UCB or an RD/1AG-MM-GVH.

MATERIALS AND METHODS

Data collection

Data for patients (age: ⩾16 years) with acute myeloid leukemia, acute lymphoblastic leukemia, MDS and chronic myelogenous leukemia who received a first HCT using a single HLA 0–2 antigen-mismatched UCB unit or an RD/1AG-MM-GVH between 1 January 1998 and 31 December 2009 were obtained from the Transplant Registry Unified Management Program (TRUMP),[24] which includes data from the Japan Cord Blood Bank Network (JCBBN) and the Japan Society for Hematopoietic Cell Transplantation (JSHCT). Our analysis included 2306 patients who received a single UCB graft (UCB group) and 541 patients who received a graft from an RD/1AG-MM-GVH (RD/1AG-MM-GVH group). As of January 2012, double UCB grafts for HCT are not available in Japan. The following patients were excluded: 26 patients who lacked data on survival status, survival date, sex of recipient, or GVHD prophylaxis and 8 patients who received stem cells that had been manipulated by *ex vivo* T-cell depletion or CD34 selection. Overall, 2288 patients who received a UCB unit and 525 who received a graft from an RD/1AG-MM-GVH fulfilled the criteria. The study was approved by the data management committees of TRUMP and by the institutional review boards of Japanese Red Cross Nagoya First Hospital and Saitama Medical Center, Jichi Medical University, where this study was organized.

Histocompatibility

Histocompatibility data for the HLA-A, HLA-B and HLA-DR loci were obtained from reports from the institution where the transplantation was performed or from cord blood banks. To reflect current practice in Japan, HLA matching in UCB or RD/1AG-MM-GVH transplantation was assessed by serological data for HLA-A, HLA-B, and HLA-DR loci. An HLA mismatch in the GVH direction was defined as when the recipient's antigens or alleles were not shared by the donor, whereas a mismatch in the HVG direction was defined as when the donor's antigens or alleles were not shared by the recipient.

End points

The primary end point of the study was to compare OS rates between the UCB and RD/1AG-MM-GVH groups. Other end points were the cumulative incidences of neutrophil and platelet engraftment, acute and chronic GVHD, relapse, and non-relapse mortality (NRM). Neutrophil recovery was considered to have occurred when the absolute neutrophil count exceeded 0.5×10^9/l for 3 consecutive days following transplantation. Platelet recovery was considered to have occurred when the absolute platelet count exceeded 50×10^9/l without platelet transfusion. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to the traditional criteria.[25,26] The incidence of chronic GVHD was evaluated in patients who survived for at least 100 days.

Statistical analysis

Descriptive statistics were used to summarize variables related to the patient characteristics. Comparisons between groups were performed with the χ^2-test or extended Fisher's exact test as appropriate for categorical variables and the Mann–Whitney *U*-test for continuous variables. The probability of OS was estimated according to the Kaplan–Meier method, and the groups were compared with the log-rank test. The adjusted probability of OS was estimated according to the Cox proportional-hazards model, with other significant variables considered in the final multivariate model. The probabilities of neutrophil and platelet engraftment, acute and chronic GVHD, NRM, and relapse were estimated on the basis of cumulative incidence methods, and the groups were compared with the Gray test;[27,28] competing events were death without engraftment for neutrophil and platelet engraftment, death or relapse without GVHD for acute and chronic GVHD, death without relapse for relapse, and relapse for NRM. The Cox proportional-hazards model was used to evaluate variables that may affect OS, whereas the Fine and Gray proportional-hazards model was used to evaluate variables that may affect engraftment, GVHD, NRM and relapse.[29] We classified the conditioning regimen as myeloablative if either total body irradiation >8 Gy, oral busulfan ⩾9 mg/kg, intravenous busulfan ⩾7.2 mg/kg, or melphalan >140 mg/m^2 was used in the conditioning regimen, and otherwise classified it as reduced intensity, based on the report by the Center for International Blood and Marrow Transplant Research.[30] For patients for whom the doses of agents used in the conditioning regimen were not available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by the treating clinicians. Acute leukemia in the first or second remission, chronic myelogenous leukemia in the first or second chronic phase or accelerated phase, and MDS with refractory anemia or refractory anemia with ringed sideroblasts were defined as standard-risk diseases, and other conditions were defined as high-risk diseases. The following variables were considered when comparing the UCB and RD/1AG-MM-GVH groups: the recipient's age group (⩽50 years or >50 years at transplantation), sex of recipient, disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia or MDS), disease status before transplantation (standard- or high-risk), type of conditioning regimen (myeloablative or reduced intensity), type of GVHD prophylaxis (calcineurin inhibitor and methotrexate, calcineurin inhibitor only, or other), year of transplantation (1998–2004, 2005–2009), and the time from diagnosis to transplantation (<6 months or ⩾6 months). In the analysis within the RD/1AG-MM-GVH group, the use of *in vivo* T cell depletion (no vs yes), stem cell source (peripheral blood (PB) stem cells vs bone marrow (BM)), and the number of HLA mismatches in the HVG direction (0–1 vs 2–3) were also considered. Factors without a variable of main interest were selected in a stepwise manner from the model with a variable retention criterion of $P<0.05$. We then added a variable of main interest to the final model. All tests were two-sided, and $P<0.05$ was considered to indicate statistical significance. All statistical analyses were performed with Stata version 12 (Stata Corp., College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[31] EZR is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria). More precisely, it is a modified version of R commander (version 1.6–3) that was designed to add statistical functions that are frequently used in biostatistics.

RESULTS

Characteristics of patients and transplants

Table 1 shows the patient and transplant characteristics. Recipients of an RD/1AG-MM-GVH were younger than recipients of a UCB unit. Approximately half of the recipients in the RD/1AG-MM-GVH group received PB. The number of HLA mismatches in the GVH direction between a UCB unit and recipient was 0 in 10%, 1 in 33% and 2 in 57%. In the RD/1AG-MM-GVH group, the number of antigen mismatches in the HVG direction was 0 in 12%, 1 in 68%, 2 in 18% and 3 in 3%. Most of the recipients of an RD/1AG-MM-GVH received a calcineurin inhibitor with methotrexate for GVHD prophylaxis, whereas 25% of UCB recipients received only calcineurin inhibitor. *In vivo* T-cell depletion including antithymocyte globulin (ATG) or alemtuzumab was used in 10% of the RD/1AG-MM-GVH group, but in only 1% of the UCB group. Alemtuzumab was used in only one patient, who received transplantation from an RD/1AG-MM-GVH. Information regarding the dose and type of ATG was missing in two-third of the patients who received ATG. Available data showed that the median dose of thymoglobulin was 2.5 (range 2.5–9.0, $n=9$) and 2.5 (range 1.25–5.0, $n=10$) mg/kg and the median dose of ATG-Fresenius was 8.0 (range 5.0–10.0, $n=3$) and 8.0 (range 5.0–10.0, $n=7$) mg/kg, in the UCB and RD/1AG-MM-GVH groups, respectively. Two-third of UCB transplantations were performed between 2005 and 2009. The median duration of follow-up for survivors was 2 and 4 years in the UCB and RD/1AG-MM-GVH groups, respectively.

Neutrophil and platelet engraftment

The incidence of neutrophil engraftment at day 50 in the RD/1AG-MM-GVH group was higher than that in the UCB group (UCB group, 73%, 95% confidence interval (CI), 71–75%; RD/1AG-MM-GVH group, 93%, 95% CI, 91–95%; Gray test, $P<0.001$; Figure 1a). The incidence of platelet engraftment at day 150 in the

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Impact of graft-versus-host disease on outcomes after allogeneic hematopoietic cell transplantation for adult T-cell leukemia: a retrospective cohort study

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Impact of graft-versus-host disease on outcomes after allogeneic hematopoietic cell transplantation for adult T-cell leukemia: a retrospective cohort study

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Allogeneic hematopoietic cell transplantation (HCT) is an effective treatment for adult T-cell leukemia (ATL), raising the question about the role of graft-versus-leukemia effect against ATL. In this study, we retrospectively analyzed the effects of acute and chronic graft-versus-host disease (GVHD) on overall survival, disease-associated mortality, and treatment-related mortality among 294 ATL patients who received allogeneic HCT and survived at least 30 days posttransplant with sustained engraftment. Multivariate analyses treating the occurrence of GVHD as a time-varying covariate demonstrated that the development of grade 1-2 acute GVHD was significantly associated with higher overall survival (hazard ratio [HR] for death, 0.65; $P = .018$) compared with the absence of acute GVHD. Occurrence of either grade 1-2 or grade 3-4 acute GVHD was associated with lower disease-associated mortality compared with the absence of acute GVHD, whereas grade 3-4 acute GVHD was associated with a higher risk for treatment-related mortality (HR, 3.50; $P < .001$). The development of extensive chronic GVHD was associated with higher treatment-related mortality (HR, 2.75; $P = .006$) compared with the absence of chronic GVHD. Collectively, these results indicate that the development of mild-to-moderate acute GVHD confers a lower risk of disease progression and a beneficial influence on survival of allografted patients with ATL. (*Blood*. 2012;119(9):2141-2148)

Introduction

Adult T-cell leukemia (ATL) is a mature T-cell neoplasm that is causally associated with a retrovirus designated human T-cell leukemia virus type I (HTLV-I).[1-4] HTLV-I is endemic in southwestern Japan, sub-Saharan Africa, the Caribbean Basin, and South America.[3,4] In Japan, more than 1 million people were estimated to be infected with HTLV-I. Although the majority of HTLV-I–infected individuals remain asymptomatic throughout their lives, ~ 5% develop ATL at a median age of 40 to 60 years.[4,5]

ATL is categorized into 4 clinical variants according to its clinical features: smoldering, chronic, acute, and lymphoma types.[6] The acute and lymphoma variants of ATL have an extremely poor prognosis, mainly because of resistance to a variety of cytotoxic agents and susceptibility to opportunistic infections; the median survival time is ~ 13 months with conventional chemotherapy,[7,8] although encouraging results have been recently reported with the use of novel agents such as mogamulizumab.[9-11]

Over the past decade, allogeneic hematopoietic cell transplantation (HCT) has been increasingly performed with the aim of improving dismal prognosis of patients who developed ATL.[12-18] Notably, some patients with ATL who relapsed after allogeneic HCT were shown to achieve remission only with the cessation of immunosuppressive agents, raising the question of whether the graft-versus-leukemia effect against ATL can be induced as part of graft-versus-host reaction.[19,20] In 1 study, among 10 patients who experienced relapse of ATL after transplantation and were withdrawn from immunosuppressive therapy, 8 developed graft-versus-host disease (GVHD), and 6 of them subsequently achieved

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Impact of the Direction of HLA Mismatch on Transplantation Outcomes in Single Unrelated Cord Blood Transplantation

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A B S T R A C T

The impact of the direction of HLA mismatch (MM) on outcome in unrelated cord blood (UCB) transplantation has not yet been clarified. We conducted a retrospective study using national registry data on 2977 patients who underwent transplantation using a single UCB for leukemia or myelodysplastic syndrome. HLA matching was assessed by serologic data for HLA-A, -B, and -DR loci. The median age of the recipients at transplantation was 41 years (range, 0-82 years), and 2300 recipients (77%) were age ≥16 years. The 2-year overall survival rate was 0.46. The presence of MM only in the graft-versus-host direction or only in the host-versus-graft direction was not associated with overall mortality (hazard ratio [HR], 0.88; $P = .317$ and HR, 0.95; $P = .670$, respectively) compared with 1 bidirectional MM. This finding was consistent in both the child and adult cohorts. The presence of MM only in the graft-versus-host direction was associated with a lower incidence of nonrelapse mortality (HR, 0.65; $P = .040$), significant only in the child cohort. No MM category was associated with relapse. Our findings suggest that the direction of HLA MM does not have a significant impact on overall survival after UCB transplantation.

INTRODUCTION

Unrelated cord blood (UCB) has emerged as a promising alternative source of hematopoietic stem cells for adult and pediatric allogeneic hematopoietic cell transplantation [1-4], and the use of UCB transplantation (UCBT) has been rapidly increasing, particularly in the United States, Europe, and Japan. One advantage of using UCB as a hematopoietic stem cell source is that UCBT requires less stringent HLA matching compared with bone marrow or peripheral blood stem cell transplantation, making it easier to find candidate UCB units in UCB banks. One or 2 antigen/allele mismatches (MMs) in the HLA-A, -B, and -DR loci between a UCB unit and recipient are acceptable without ex vivo T cell depletion methods, and the clinical outcome of transplantation using a 0-2 antigen/allele-mismatched UCB unit was almost comparable to that from an HLA allele-matched unrelated donor [1-3].

Although the number of HLA MMs between a UCB unit and a recipient is usually counted without considering the MM direction, the effect of the immune reaction caused by HLA MM differs according to whether the MM is in the graft-versus-host (GVH) or host-versus-graft (HVG) direction. A mismatched antigen in the GVH direction can be a major target for donor T cells and can cause graft-versus-host disease (GVHD), whereas a mismatched antigen in the HVG direction can be a major target for the remaining recipient T cells and can lead to graft rejection. In related transplantation, the presence of HLA MMs in the GVH direction is associated with a higher incidence of GVHD, whereas the

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presence of HLA MMs in the HVG direction is associated with a higher incidence of rejection [5-7]. Therefore, from a biological perspective, the impact of HLA MM should be discussed separately according to the direction of MM. However, because most patients have an equal number of MMs in the GVH and HVG directions (bidirectional MM), studying an adequate number of patients to evaluate an MM imbalance in the GVH and HVG directions has proven difficult.

The few studies that have evaluated the impact of the HLA MM direction on UCBT outcome have reported inconsistent results [8-10]. Matsuno et al. [8] reported that an HLA MM in the GVH direction was associated with lower incidence of neutrophil engraftment. In contrast, Stevens et al. [9] showed that UCBT with an MM only in the GVH direction was associated with a lower incidence of nonrelapse mortality (NRM) and overall mortality compared with UCBT with an 1 bidirectional MM, whereas UCBT with an MM only in the HVG direction was associated with a lower incidence of neutrophil engraftment and a higher incidence of relapse.

To clarify the significance of the direction of HLA MM on transplantation outcomes, we conducted a retrospective study using national registry data in 2977 patients who underwent a single UCBT.

METHODS

Data Collection

Data for 2987 patients with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), and chronic myelogenous leukemia (CML) who underwent a first transplantation using a single UCB unit between January 1, 1998, and December 31, 2009, were obtained from the Transplant Registry Unified Management Program (TRUMP) [11], in which all UCBTs are registered through the Japan Cord Blood Bank Network (JCBBN), a national network of all 11 cord blood banks in Japan. Ten patients lacking data on survival status or survival date were excluded. A total of 2977 patients met the criteria for study inclusion. The study design was approved by the TRUMP Data Management Committee and the Institutional Review Board of Saitama Medical Center, Jichi Medical University, where this study was organized.

Histocompatibility

Histocompatibility data for the HLA-A, -B, and -DR loci were obtained from reports collected from the institution at which the transplantation was performed or cord blood banks. HLA typing methods have been described previously [12]. To reflect current practice in Japan, HLA matching was assessed by serologic data for HLA-A, -B, and -DR loci. A secondary analysis using antigen level data for HLA-A, -B and available allele level data for HLA-DRB1 was also performed to compare our data with previously published data from the United States and Europe. HLA-DRB1 allele information was available in 84% of patients (2498 of 2977). Among these patients, 62% had the same number of MMs at HLA-DRB1 loci at either the antigen or allele level. An HLA MM in the GVH direction was defined as when the recipient's antigens or alleles were not shared by the donor, and an MM in the HVG direction was defined as when the donor's antigens or alleles were not shared by the recipient.

Endpoints

The primary study endpoint was overall survival (OS). Other endpoints assessed were relapse, NRM, neutrophil and platelet engraftment, grade II-IV or III-IV acute GVHD, and chronic GVHD. Neutrophil recovery was defined as an absolute neutrophil count exceeding 0.5×10^9/L for 3 consecutive days after UCBT. Platelet recovery was defined as an absolute platelet count exceeding 50×10^9/L without platelet transfusion. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to traditional criteria [13,14]. The incidence of acute GVHD was evaluated in patients who engrafted, and that of chronic GVHD was evaluated in patients who engrafted and survived for more than 100 days.

Statistical Analysis

The probability of OS was estimated according to the Kaplan–Meier method and the groups were compared using the log-rank test. The probabilities of relapse, NRM, neutrophil and platelet engraftment, and acute and chronic GVHD were estimated based on cumulative incidence curves [15]. Competing events were death without relapse for relapse, relapse for NRM, death without engraftment for neutrophil and platelet engraftment, and death or relapse without GVHD for acute and chronic GVHD. The groups were compared using Gray's test [16]. The Cox proportional hazards model was used to evaluate the effect of confounding variables on OS, and the Fine and Gray proportional hazards model was used for the other endpoints [17]. Based on the report by the Center for International Blood and Marrow Transplant Research, we classified the conditioning regimens as myeloablative if total body irradiation >8 Gy, oral busulfan ≥9 mg/kg, i.v. busulfan ≥7.2 mg/kg, or melphalan >140 mg/m^2 was used in the conditioning regimen; otherwise, the conditioning regimen was classified as reduced intensity [18]. For patients with insufficient data regarding dosages of the agents used in the conditioning regimen, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by the treating clinicians. We defined AML and ALL in first or second remission, CML in first or second chronic phase or accelerated phase, and MDS with refractory anemia or refractory anemia with ringed sideroblasts as standard risk, and all other conditions as high risk.

The following possible confounding variables were considered: recipient age group (0-5 years, 6-15 years, 16-49 years, or ≥50 years at transplantation), matching of ABO blood type between the recipient and UCB (match or major, minor, or bidirectional MM), recipient sex, sex MM between recipient and UCB (match, male donor–female recipient, or female donor–male recipient), disease (AML, ALL, CML, or MDS), disease status before transplantation (standard or high risk), type of conditioning regimen (myeloablative or reduced intensity), type of GVHD prophylaxis (calcineurin inhibitor plus methotrexate, calcineurin inhibitor only, others), and year of transplantation (1998-2004 or 2005-2009). Factors other than HLA MM and total nucleated cell (TNC) dose category were selected in a stepwise manner from the model with a variable retention criterion of $P < .05$. HLA MM and TNC dose category (≥10.0, 5.0-9.9, 2.5-4.9, 2.0-2.4, and $<2.0 \times 10^7$/kg) were then added to the final model. All tests were 2-sided, and a P value <.05 was considered statistically significant. All statistical analyses were performed with Stata version 12 (StataCorp, College Station, TX) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria) [19]. More precisely, EZR is a modified version of R commander (version 1.6-3) designed to add statistical functions used frequently used in biostatistics.

RESULTS

Patient Characteristics

Table 1 summarizes patient and transplant characteristics. The median age of the recipients at transplantation was 41 years (range, 0-82 years), and 2300 patients (77%) were age ≥16 years. Diagnoses for transplantation were AML in 1606 patients, ALL in 893, CML in 135, and MDS in 343. Half of the patients had standard-risk disease. UCBT was performed between 1998 and 2004 in 1153 patients (39%) and between 2005 and 2009 in 1824 patients (61%). The combination of a calcineurin inhibitor (tacrolimus or cyclosporine) and methotrexate was used in 62% of patients, whereas a calcineurin inhibitor alone was used in 22% of patients.

Some 40% of patients received a UCB unit containing $<2.5 \times 10^7$/kg TNCs, and 45% received a UCB unit containing $2.5\text{-}4.9 \times 10^7$/kg TNCs. Roughly 12% of patients received $\geq 5.0 \times 10^7$/kg TNCs, but 93% of these patients were age <16 years. Median body weight was 17 kg (range, 4-68 kg) for the children and 55 kg (range, 24-165 kg) for the adults. HLA MM was categorized as follows: HLA match in both the GVH and HVG directions (GVH 0/HVG 0 MM group; n = 273 [9%]), 1-2 antigen MMs in the GVH direction but 0 MMs in the HVG direction (GVH 1-2/HVG 0 MM group; n = 150 [5%]), 1-2 antigen MMs in the HVG direction but 0 MM in the GVH direction (GVH 0/HVG 1-2 MM group; n = 136 [5%]), 1 antigen MM in both the GVH and HVG directions at the same locus (GVH 1/HVG 1 MM group; n = 716 [24%]), 2 antigen MMs in both the GVH and HVG directions (GVH 2/HVG 2 MM group; n = 1170 [39%]), 2 antigen MMs in the GVH direction and 1 antigen MM in the HVG direction (GVH 2/HVG 1 MM group; n = 231 [8%]), 1 antigen MM in the GVH direction and

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Cells in focus

Bone marrow stromal cells (bone marrow-derived multipotent mesenchymal stromal cells) for bone tissue engineering: Basic science to clinical translation

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ABSTRACT

Bone tissue engineering is a promising field of regenerative medicine in which cultured cells, scaffolds, and osteogenic inductive signals are used to regenerate bone. This technology has already been used in several clinical studies and its efficacy has been reported. In this review, we focus on bone marrow stromal cells, which are the most commonly used cell source for bone tissue engineering. The nature of the cells, suitable culture conditions for bone tissue engineering, and their potential therapeutic applications are reviewed with possible caveats. Furthermore, recent advances in bone marrow stromal cell biology are discussed with reference to clinical translation.

Cell facts

- Bone marrow stromal cells (bone marrow-derived mesenchymal stromal cells) are also designated mesenchymal stem cells, but indeed the majority of the cells are not true stem cells.
- The differentiative potential of bone marrow stromal cells is unstable during culture and the cells lose their plasticity with continued proliferation.
- Bone marrow stromal cells are, however, still considered as a most reliable and clinically confirmed cell source for bone tissue engineering.

1. Introduction

Although the presence of osteogenic cells in bone marrow has long been known (Friedenstein et al., 1966), the detailed nature of those cells has been described only recently. The presence of adherent fibroblast-like cells in bone marrow aspirates was also reported by Friedenstein et al. (1970). Since the culture contains cells which can generate single cell-derived colonies, those cells were referred to as colony forming units-fibroblasts (CFUs-F). CFUs-F possess high proliferating potential and even single-colony derived cells can form bone. Accordingly, not all but some CFUs-F can be considered as osteogenic stem/progenitor cells (Friedenstein et al., 1987). Subsequently, the capability of CFUs-F to differentiate to various mesenchymal tissues has been reported, which leaded to the concept of mesenchymal stem cells (MSCs) (Caplan, 1991).

Although the adherent cells from bone marrow have been used for bone tissue engineering, the population is heterogeneous and only a limited number of cells (putative stem/progenitor cells) can form a secondary (osteogenic) colony (Sacchetti et al., 2007). This fact, together with the lack of appropriate markers, has created a confusing nomenclature (Bianco et al., 2008; Prockop, 2009). The cells have been called both CFUs-F and MSCs but also bone marrow stromal cells and mesenchymal stromal cells. Although the pluripotency of (a proportion of) those adherent cells has been repeatedly confirmed at the single cell-derived colony level, not all cells are multipotent and the culture contains cells with a range of differentiation status (Mizuno et al., 2010). Accordingly, we prefer to call the cells "bone marrow stromal cells (BMSCs)", which is a relatively widely used nomenclature (Prockop, 2009). Since multipotent cells have been isolated from various mesenchymal tissues

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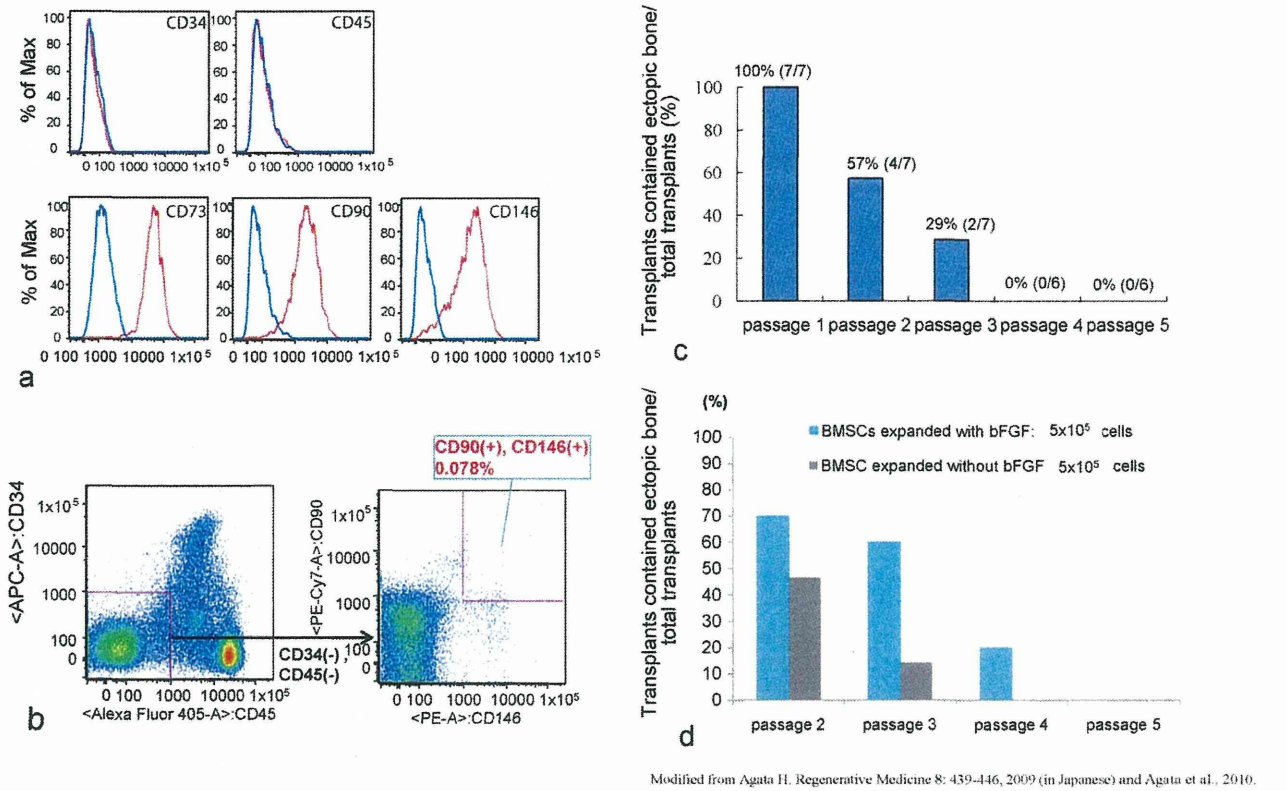

Modified from Agata H. Regenerative Medicine 8: 439-446, 2009 (in Japanese) and Agata et al., 2010.

Fig. 1. Characteristics of BMSCs. (a) Cell surface marker analyses of cultured BMSCs by flow cytometry. (b) Flow cytometric analysis of whole bone marrow cells. Only 0.078% of whole marrow cells are CD34(-), CD45(-), CD90(+) and CD146(+), which are the reported markers (but not specific) for BMSCs. (c) The percentage of successful ectopic bone formation with two week-induced BMSCs. (d) *In vivo* osteogenic ability of BMSCs expanded with or without bFGF.
This figure was modified from the original figure in Agata H. Establishment of truly reliable protocol for bone tissue engineering. Regenerative Medicine 8: 439–446, 2009 (in Japanese) with permission.

including bone marrow, The International Society for Cellular Therapy has suggested calling the cells "multipotent mesenchymal stromal cells". Thus, BMSCs can also be called bone marrow-derived multipotent mesenchymal stromal cells (MSCs) according to the suggestion of Horwitz et al. (2005).

There is no established, widely accepted marker for osteogenic stem/progenitor cells in bone marrow. The International Society for Cellular Therapy has provided the minimum criteria for defining MSCs which are positive for CD105, CD73 and CD90 and negative for CD34, CD45, CD11a, CD19 and HLA-DR (Dominici et al., 2006). However, those markers are not sufficient to define a specific population for osteogenic stem/progenitor cells. A recent study has demonstrated MCAM/CD146$^+$ cells might be a reasonable candidate (Sacchetti et al., 2007) (Fig. 1a). Other than CD146, many other cell surface markers have been proposed, such as CD271, mesenchymal stem cell antigen-1 (MSCA-1), CD56, SSEA-4, STRO-1, and platelet-derived growth factor receptor-beta (PDGF-RB; CD140b) to enrich the population (reviewed by Bernardo et al., 2009; Salem and Thiemermann, 2010).

2. Cell origin and plasticity

Since the BMSC population is heterogeneous, it is difficult to define a single origin for all the cells. In human bone marrow, CD146$^+$ cells, which are candidates for osteogenic stem/progenitor cells, are restricted to adventitial reticular cells (ARCs) and form a subendothelial (adventitial) layer in the sinusoidal wall (Sacchetti et al., 2007). This distribution might be a common feature of other MSCs known to be perivascular cells (Crisan et al., 2009).

BMSCs can be obtained from a patient's own bone marrow aspirate. However, the percentage of putative stem cells in BMSCs in whole bone marrow is considered as less than 0.01% (Pittenger et al., 1999) (Fig. 1b). For that reason, *in vitro* cell expansion is required for clinical application. For example, bone marrow from a single rat can provide a sufficient number of osteogenic cells for only two or three bone-forming transplants. In contrast, after *in vitro* expansion of cells from the same volume of bone marrow, more than 50 bone-forming transplants are possible (Yoshikawa et al., 1996).

Importantly, BMSCs should be appropriately cultured and induced into osteogenic cells, since their ability to differentiate into osteoblast-like cells is easily diminished during culture and passage (Sugiura et al., 2004; Agata et al., 2010). Although a number of studies of bone tissue engineering using BMSCs have been published, it is surprising that there is no established and widely accepted cell culture/induction protocol. One of the reasons for this confusion might be the complex nature of optimization processes. For successful tissue engineering, various factors should be investigated and optimized, i.e., cell separation procedures, culture media, concentration, type and origin of sera, cell seeding density, timing of passaging, number of passages, content and concentrations of reagents for induction, the period of induction. Also important are the shape, size, and material used for scaffolds, and the time at which the cells are seeded on scaffolds. The culture conditions should be optimized depending on the ultimate clinical purpose, since BMSCs consist of mixed populations and each culture condition may preferentially support the growth of one or some of those subpopulations.

We have tried to optimize culture protocols for bone tissue engineering with human BMSCs and granular type β-tricalcium phosphate (β-TCP) scaffold using an ectopic transplantation model,

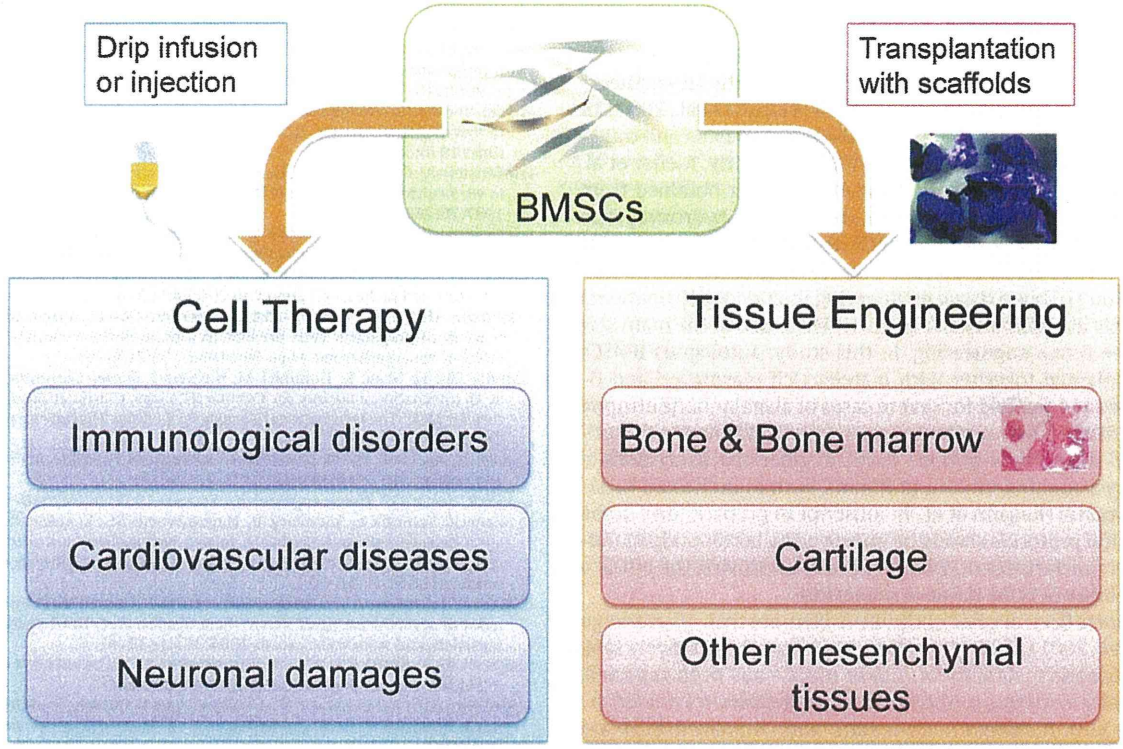

Fig. 2. Representative functions of BMSCs.

which is considered the most reliable model for characterizing the osteogenic ability of the cells. The results showed that the passage number, seeding density and the period of induction significantly affects the cells' osteogenic ability. Surprisingly, adult human BMSCs lose their *in vivo* bone forming ability very rapidly and no bone formation was observed after the third passage (Fig. 1c) (Agata et al., 2010). Although there is no known mechanism for this characteristic change during passage, our study showed beneficial effects of basic fibroblast growth factor (bFGF) to maintain osteogenic ability of BMSCs (Fig. 1d) (Agata et al., 2010). A controversy exists regarding the use of dexamethasone, which has been a widely accepted and proven osteogenic induction factor for human and most other mammalian BMSCs (McCulloch and Tenenbaum, 1986; Grigoriadis et al., 1988). Although ectopic bone formation is feasible with non-induced BMSCs under certain circumstances, the results from recent investigations demonstrated that osteogenic induction with dexamethasone is required for BMSCs with relatively low osteogenic ability (e.g., elderly patients) (Mendes et al., 2002). Thus, osteogenic induction may increase the probability of *in vivo* bone formation, and contribute to the reliability of bone tissue engineering.

3. Functions

BMSCs are commonly used as a source of cells for tissue engineering. Tissue engineering seeks to regenerate tissue using cells, scaffold and bioactive molecules (Langer and Vacanti, 1993). One of the critical functions of BMSCs under physiological conditions is to support the process of bone remodeling by providing osteoblasts. In addition, BMSCs may play an important supportive role for bone marrow, since recent findings have suggested that osteoblasts provide a niche for hematopoietic stem cells (HSCs) and can modulate the state of HSCs from quiescence to active phase for hematopoiesis (reviewed by Garrett and Emerson, 2009). Therefore, one of the important physiological functions of BMSCs is to support the homeostasis of bone and bone marrow. In part, this function is maintained after expansion *in vitro*, which has been used for bone tissue engineering (Fig. 2). Currently, bone tissue engineering using BMSCs has been applied clinically and shows promising results (Kagami and Agata, 2010). BMSCs also differentiate into chondrogenic and adipogenic cells, which might represent additional normal physiological functions.

Although one of the important physiological functions of BMSCs seems to be the maintenance of bone homeostasis as stated above, they may have more functions. BMSCs have been directly injected to a site of disease or infused intravenously like a systemic pharmacologic agent. In this review, this approach is called "cell therapy" in contrast with "tissue engineering" (Fig. 2). For cell therapy, BMSCs play an important role in immunomodulation. BMSCs suppress proliferation of allogeneic lymphocytes (Le Blanc et al., 2003), a property which has been used to reduce the incidence and severity of graft-versus-host disease (GVHD) during allogeneic transplantation (Le Blanc et al., 2004). The immunosuppressive effect of BMSCs was well established from *in vitro* studies. However, the function of BMSCs may be modulated by the microenvironmental context, thus *in vivo* biological relevance requires further clarification (reviewed by Aronin and Tuan, 2010).

Other than immunomodulatory/immunoregulatory functions, additional roles for BMSCs have been proposed. BMSCs have been repeatedly investigated to determine their effects on cardiovascular diseases and neuronal damage (reviewed by Bernardo et al., 2009; Delcroix et al., 2010). Although the efficacy of BMSC cell therapy has been reported, the actual functions of BMSCs *in vivo* are still largely unknown. Direct differentiation at the site of tissue damage may not be a primary mechanism. Instead, secretion of various trophic factors/cytokines and other indirect mechanisms may contribute to their efficacy. Future studies may unveil more and even unexpected functions of the cells.

4. Associated pathologies

The results from clinical studies of alveolar bone tissue engineering with BMSCs were first reported in 2004 (Yamada et al., 2004). In this study, BMSCs were mixed with platelet rich plasma and transplanted to an atrophic maxilla or mandible. Recently, Meijer et al. (2008) reported that bone marrow aspirates were obtained from patients and BMSCs were cultured and seeded on hydroxyapatite granules. Over the following week, osteogenic cells were transplanted to sinus floor and alveolar ridge. Our group also initiated a clinical study of bone tissue engineering in 2004 for the patients with severely atrophic alveolar bone, which is one of the main targets of bone tissue engineering. In this study, autologous BMSCs were transplanted together with platelet rich plasma gel and β-TCP granules as a scaffold for severe cases of alveolar bone atrophy which required bone transplantation. The results from those studied showed the efficacy of bone tissue engineering using BMSCs, though they also showed some problems, such as individual variations in response (Kagami et al., manuscript in preparation). In the future, clinical protocols should be improved by better understanding of the characteristics of BMSCs, thereby improving the efficacy and the stability of bone tissue engineering.

BMSCs have been applied clinically from more than 10 years ago (Quatro et al., 2001). Their clinical use appears to be relatively safe. However, genetic instability of mouse BMSCs has been reported, which readily undergo multistage carcinogenesis (reviewed by Prockop, 2009). Although such results with human BMCSs are unlikely, long-term *in vitro* cell culture may affect genomic stability. Since there is no established method to detect genetic alteration during culture, limiting the number of passages (population doublings) is a sensible precaution. Quest for more potent somatic stem cells may also be accompanied by greater risk of genetic change. These limitations should be kept in mind in future clinical studies and therapeutic applications.

References

Agata H, Asahina I, Watanabe N, Ishii Y, Kubo N, Ohshima S, et al. Characteristic change and loss of *in vivo* osteogenic abilities of human bone marrow stromal cells during passage. Tissue Eng Part A 2010;16:663–73.

Aronin CEP, Tuan RS. Therapeutic potential of the immunomodulatory activities of adult mesenchymal stem cells. Birth Defects Res (Part C) 2010;90:67–74.

Bernardo ME, Locatelli F, Fibbe WE. Mesenchymal stromal cells. A novel treatment modality for tissue repair. Hematopoietic stem cells VII. Ann N Y Acad Sci 2009;1176:101–17.

Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cell: revisiting history, concepts, and assays. Cell Stem Cell 2008;2:313–9.

Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9:641–50.

Crisan M, Yap S, Casteilla L, Chen C-W, Corselli M, Park TS, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2009;3:301–13.

Delcroix GJ-R, Schiller PC, Benoit J-P, Montero-Menei CN. Adult cell therapy for brain neuronal damages and the role of tissue engineering. Biomaterials 2010;31:2105–20.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–7.

Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 1970;3:393–403.

Friedenstein AJ, Challakhyan RK, Gerasimov UV. Bone marrow osteogenic stem cells: *in vitro* cultivation and transplantation in diffusion chambers. Cell Tissue Kinet 1987;20:263–72.

Friedenstein AJ, Piatetzky-Chapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morph 1966;16:381–90.

Garrett RW, Emerson SG. Bone and blood vessels: the hard and the soft of hematopoietic stem cell niches. Cell Stem Cell 2009;4:503–6.

Grigoriadis AE, Heersche JNM, Aubin JE. Differentiation of muscle, fat, cartilage, and bone from progenitor cells present in a bone-derived clonal cell population: effect of dexamethasone. J Cell Biol 1988;106:2139–51.

Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, et al. International Society for Cellular Therapy. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy 2005;7:393–5.

Kagami H, Agata H. The potential of somatic stem cells for alveolar bone tissue engineering. Int J Oral-Med Sci 2010;9:1–10.

Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–6.

Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringdén O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol 2003;57:11–20.

Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004;363:1439–41.

Meijer GJ, de Bruijn JD, Koole R, van Blitterswijk CA. Cell based bone tissue engineering in jaw defects. Biomaterials 2008;29:3053–61.

McCulloch CAG, Tenenbaum HC. Dexamethasone induces proliferation and terminal differentiation of osteogenic cells in tissue culture. Anat Rec 1986;215:397–402.

Mendes SC, Tibbe JM, Veenhof M, Bakker K, Both S, Platenburg PP, et al. Bone tissue-engineered implants using human bone marrow stromal cells: effect of culture conditions and donor age. Tissue Eng 2002;8:911–20.

Mizuno M, Agata H, Furue H, Kimura A, Narita Y, Watanabe N, et al. Limited but heterogeneous osteogenic response of human bone marrow mesenchymal stem cells to bone morphogenetic protein-2 and serum. Growth Factors 2010;28:34–43.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.

Prockop DJ. Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. Mol Ther 2009;17:939–46.

Quatro R, Mastrogiacomo M, Cancedda R. Repair of large bone defects with the use of autologous bone marrow stromal cells. N Engl J Med 2001;344:385–6.

Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, et al. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 2007;131:324–36.

Salem HK, Thiemermann C. Mesenchymal stromal cells: current understanding and clinical status. Stem Cells 2010;28:585–96.

Sugiura F, Kitoh H, Ishiguro N. Osteogenic potential of rat mesenchymal stem cells after several passages. Biochem Biophys Res Commun 2004;26:233–9.

Yamada Y, Ueda M, Hibi H, Nagasaka T. Translational research for injectable tissue-engineered bone regeneration using mesenchymal stem cells and platelet-rich plasma: from basic research to clinical case study. Cell Transplant 2004;13:343–55.

Yoshikawa T, Ohgushi H, Tamai S. Immediate bone forming capability of prefabricated osteogenic hydroxyapatite. J Biomed Mater Res 1996;32:481–92.