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RESEARCH ARTICLE

Safety of autologous bone marrow-derived mesenchymal stem cell transplantation for cartilage repair in 41 patients with 45 joints followed for up to 11 years and 5 months

Shigeyuki Wakitani[1]*, Takahiro Okabe[1], Shuji Horibe[2], Tomoki Mitsuoka[2], Masanobu Saito[3], Tsuyoshi Koyama[3], Masashi Nawata[4], Keiji Tensho[4], Hiroyuki Kato[4], Kota Uematsu[5], Ryosuke Kuroda[6], Masahiro Kurosaka[6], Shinichi Yoshiya[7], Koji Hattori[8] and Hajime Ohgushi[8]
[1]*Department of Orthopaedic Surgery, Osaka City University Graduate School of Medicine, Japan*
[2]*Department of Orthopaedic Surgery, Osaka Rosai Hospital, Japan*
[3]*Department of Orthopaedic Surgery, National Hospital Organization, Osaka Minami Medical Centre, Japan*
[4]*Department of Orthopaedic Surgery, Shinshu University, Japan*
[5]*Department of Orthopaedic Surgery, Nara Medical University, Japan*
[6]*Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, Japan*
[7]*Department of Orthopaedic Surgery, Hyogo College of Medicine, Japan*
[8]*Research Institute for Cell Engineering, Advanced Industrial Science and Technology, Amagasaki site, Kansai, Japan*

Abstract

Among autologous somatic stem cells, bone marrow-derived mesenchymal stem cells (BMSCs) are the most widely used worldwide to repair not only mesenchymal tissues (bone, cartilage) but also many other kinds of tissues, including heart, skin, and liver. Autologous BMSCs are thought to be safe because of the absence of immunological reaction and disease transmission. However, it is possible that they will form tumours during long-term follow-up. In 1988, we transplanted autologous BMSCs to repair articular cartilage, which was the first such trial ever reported. Subsequently we performed this procedure in about 40 patients. Demonstration that neither partial infections nor tumours appeared in these patients provided strong evidence for the safety of autologous BMSC transplantation. Thus, in this study we checked these patients for tumour development and infections. Between January 1998 and November 2008, 41 patients received 45 transplantations. We checked their records until their last visit. We telephoned or mailed the patients who had not visited the clinics recently to establish whether there were any abnormalities in the operated joints. Neither tumours nor infections were observed between 5 and 137 (mean 75) months of follow-up. Autologous BMSC transplantation is a safe procedure and will be widely used around the world.

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1. Introduction

Obtaining cells for transplantation is a major issue in tissue engineering and regenerative medicine. It is possible to transplant allogeneic cells, but these are not widely used in clinical practice because of the likelihood of immunological reactions and disease transmission (Wakitani *et al*., 1989). Autologous cell transplantation is another possibility. This is better suited to clinical practice because there is neither immunological reaction nor disease transmission, but an important problem is the limited amount of tissue that can be collected from the patient, because of donor site morbidity (Ochi *et al*.,

**Correspondence to: Shigeyuki Wakitani, Department of Orthopaedic Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi Abeno-ku, Osaka 545-8585, Japan. E-mail: wakitani@med.osaka-cu.ac.jp*

2002). Among the types of autologous cells that may be used in transplantation, somatic stem cells are the most suitable because of their simultaneous capacity for proliferation and differentiation. Among autologous somatic stem cells, bone marrow-derived mesenchymal stem cells (BMSCs) are the most used worldwide because they can be collected easily without producing tissue defect (Wakitani *et al*., 1994). When bone marrow blood is cultured in a plastic tissue, adherent cells appear and proliferate. These cells were first described 40 years ago as progenitor cells of bone and cartilage (Friedenstein *et al*., 1966; Ohgushi *et al*., 1989). In the 1990s these cells were reported to differentiate into muscle cells and adipocytes and were called BMSCs because they are derived from mesoderm (Caplan, 1991; Wakitani *et al*., 1995; Johnstone *et al*., 1998; Pittenger *et al*., 1999). In 1999 these cells were reported to differentiate into nerve cells, which are normally derived from ectoderm (Kopen *et al*., 1999), and hepatocytes, normally derived from endoderm (Petersen *et al*., 1999). This phenomenon was named 'transdifferentiation'. Thus, BMSCs are considered to be a source not only of mesenchymal tissues but also of many other kinds of tissue, including heart, skin and liver (Dai *et al*., 2009; Ma *et al*., 2009; van der Bogt *et al*., 2009).

Autologous BMSCs are safe because their use does not lead to either immunological reactions or disease transmission. However, it is possible for them to produce tumours during a long follow-up period. Transplantations of these cells into immunodeficient animals were performed, and many of them showed no evidence of tumour formation (Bernardo *et al*., 2007) but few papers have shown the possible potential (Rubio *et al*., 2005; Rosland *et al*., 2009). Therefore, the possibility of tumour development cannot be rejected completely.

In 1998, we transplanted autologous BMSCs to repair articular cartilage, which was the first such clinical trial ever reported from anywhere in the world (Wakitani *et al*., 2004). Subsequently we performed this procedure in about 40 patients (Wakitani *et al*., 2002, 2007; Kuroda *et al*., 2007). If we were able to show that there were no partial infections and no tumours in these patients, this would strongly support the safety of autologous BMSC transplantation. In this study we therefore checked these patients for tumour development and infections.

2. Patients and methods

We examined patients in Japan who had received autologous BMSC transplantation to repair articular cartilage. So far as we knew, 41 patients existed. Transplantation was performed in the Departments of Orthopaedic Surgery, Osaka Rosai Hospital, National Hospital Organization, Osaka Minami Medical Centre, Shinshu University, Nara Prefectural Medical University, Kobe University Graduate School of Medicine and Hyogo Medical University (Table 1). The studies were approved by the institutional review board of each institution and were in accordance with the Declaration of Helsinki of 1975, as revised in 1983.

2.1. Cell transplantation procedure

Until 2000, cells were cultured in a conventional cell culture room, located in a laboratory of the National Hospital Organization, Osaka Minami Medical Centre. The culture room was equipped with a clean bench and an incubator and separated from other laboratory areas. The transplantations of the cultured BMSCs to the patients were performed in the surgery room of the same institute or at Osaka Rosai Hospital. The procedure was described in detail previously (Wakitani *et al*., 2002, 2004). In brief, about 10 ml heparinized bone marrow blood was aspirated from the left iliac crest. In the cell culture room, erythrocytes were removed using dextran, and the remaining nucleated cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal calf serum (FCS). After approximately 10 days, the attached cells achieved subconfluence and were then passaged for expansion in culture. After another 10 days, on the day before surgery, the cells were collected and embedded in 0.25% acid-soluble type I collagen from porcine tendon (Nitta Gelatin Inc., Osaka, Japan) and gelated, or placed on a collagen sheet (Koken Inc., Tokyo, Japan) and gelated. This gel–cell composite was then cultured overnight in DMEM supplemented with 15% autologous serum.

Since 2003, cell cultures have been performed at the Cell Processing Ccentre (CPC) of the National Institute of Advanced Industrial Science and Technology, Amagasaki City. Transplantation was performed in the surgery rooms of the other institutes, which include Shinshu University, Nara Prefectural Medical University (patient NPU1), Kobe University Graduate School of Medicine and Hyogo Medical University. For the four patients NPU2–5, cell culture was performed in the CPC of Nara Prefectural Medical University. Details of the procedure were described previously (Kuroda *et al*., 2007; Wakitani *et al*., 2007). In brief, bone marrow blood was carried to the CPC, where there were three clean rooms of class 1000, and culture was started within 6 h. At the CPC, cells from 3 ml fresh marrow were placed in two 75 cm^2 plastic culture flasks. When the culture medium was changed, non-adherent haematopoietic cells were removed, leaving only adherent cells in the dish. After about 10 days, the number of adherent cells had reached several millions. The cells were collected by trypsinization (first passage) and further cultured (subcultured) in additional flasks for approximately 10 days. The culture medium used was α-minimum essential medium (α-MEM) supplemented with 15% autologous serum. Subcultured cells were collected and embedded in 1% acid-soluble type I collagen from bovine skin (Koken) (final cell density, 5×10^6 cells/ml), placed onto a collagen sheet from porcine tendon (Gunze, Kyoto, Japan) and gelated. This gel–cell composite was further cultured for 2 days. We used

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Table 1. List of patients who received autologous bone marrow MSC transplantation to repair cartilage defects

No.	Date of surgery	Patient	Joint	Disease	Age at surgery (years)	Last follow-up (source)	End-point	Follow-up (months)
1	Jan-98	ORH1	K	CD	26	Jun-09 (p)		137
2	Apr-98	OMMC1	K	OA	71	Apr-09 (p)		132
3	May-98	OMMC2-1	K(rt)	OA	69	Jun-09 (e)		133
4	Jun-98	OMMC3	K	OA	68	Jun-09 (p)		132
5	Jun-98	OMMC4	K	OA	54	Jun-09 (p)		132
6	Jul-98	OMMC5	K	OA	74	Jan-01	TKR	30
7	Aug-98	OMMC6	K	OA	64	Jun-09 (e)		130
8	Sep-98	OMMC7	K	OA	62	Jun-08	TKR	117
9	Sep-98	OMMC8	K	OA	67	Feb-02 (e)	Dead (May-02)	41
10	Oct-98	OMMC9	K	OA	65	May-00	Lost	19
11	Nov-98	OMMC10	K	OA	66	Aug-07	Lost	105
12	Nov-98	OMMC11	K	OA	68	Sep-02	TKR	46
13	Feb-99	OMMC12	K	OA	70	Mar-09 (e)		121
14	May-99	OMMC13	K	OA	65	Mar-09 (e)		118
15	Aug-99	OMMC14-1	K(lt)	OA	65	Jun-09 (e)		118
16	Oct-99	OMMC15	K	OA	55	Jun-09 (e)		116
17	Nov-99	ORH2	K	CD	44	Jun-09 (p)		115
18	Dec-99	OMMC2-2	K(lt)	OA	70	Jun-09 (e)		114
19	May-00	OMMC14-2	K(rt)	OA	66	Jun-09 (e)		109
20	Jun-00	OMMC18	K	OA	49	Jun-09 (e)		108
21	Jul-00	OMMC19	K	OA	54	Apr-09 (e)		105
22	Sep-00	OMMC20	K	OA	52	Oct-02	TKR	25
23	Oct-00	OMMC21	K	OA	60	Mar-05 (e)	Dead (Mar-05)	53
24	Oct-00	OMMC22	K	OA	67	Feb-07 (e)	Dead (Feb-09)	76
25	Oct-00	OMMC23	K	OA	66	Jul-07	TKR	81
26	Dec-00	OMMC24	K	OA	64	Jul-09 (p)		103
27	Feb-03	NMU1	A	CD	42	Feb-09 (e)		72
28	Dec-03	SU1	E	CD	13	May-09 (e)		65
29	May-04	SU2-1	K(lt)	CD	31	Jun-09 (p)		61
30	Jun-04	SU3	H	OA	38	Jun-09 (p)		60
31	Jul-04	SU4	K	OA	50	May-09 (e)		58
32	Oct-04	KU	K	CD	31	Jul-09 (e)		57
33	Dec-04	SU2-2	K(rt)	CD	31	Jun-09 (p)		54
34	Feb-05	SU5	E	CD	14	Apr-09 (e)		50
35	Feb-05	SU6	K(bil)	CD	42	Jun-09 (p)		52
36	Apr-05	SU7	K	CD	45	May-09 (e)		49
37	Aug-05	SU8	E	CD	14	May-09 (e)		45
38	May-06	NMU2	K	CD	23	Dec-07 (e)		19
39	Jun-06	NMU3	K	CD	25	May-08 (e)		23
40	Aug-06	SU9	E	CD	13	May-09 (e)		33
41	Aug-06	NMU4	K	CD	63	Sep-08 (e)		25
42	Dec-06	HCM	K	CD	39	Jun-09 (e)		30
43	May-07	NMU5	K	CD	27	Dec-08 (e)		19
44	Nov-08	NMU6	K	CD	40	Apr-09 (e)		5
	Mean				50			75

ORH, Osaka Rosai Hospital; OMMC, Osaka Minami Medical Centre; NMU, Nara Medical University; SU, Shinshu University; KU, Kobe University; HCM, Hyogo College of Medicine.
K, knee; H, hip; E, elbow; A, ankle; OA, osteoarthritis; CD, cartilage defect; (source), source of information; e, examination; p, telephone; TKR, total knee replacement; rt, right; lt, left; bil, bilateral.
Case 35 transplantation was performed in bilateral knees, simultaneously.

a conventional culture method to detect bacterial and fungal contamination, but also used the BacT/ALERT 3D Microbial Detection System (bioMerieux, Durham, NC, USA). We did contamination checks at the beginning of the culture, when we prepared the culture medium with the patient's serum, and lastly at the time of the final medium change. After confirming that there was no contamination, the composite was carried back to the hospital and transplanted (Figure 1).

2.2. Patients

Between January 1998 and November 2008, 41 patients received 45 transplantations (Table 1). Mean age at the time of surgery was 50 years. We checked the patients' records until their last visit. We telephoned or mailed the patients who had not visited recently to ask them to visit us. In the case of patients who visited us, we examined their knees and X-ray films. In cases who could not visit us, we asked them by telephone about knee function, pain and swelling in the operated knee (Table 1).

3. Results

We were unable to contact two patients. Three patients died at the age of 71, 65 and 75 years, respectively. In five patients, total knee replacement (TKR) was performed on the transplanted knee because of the progression of

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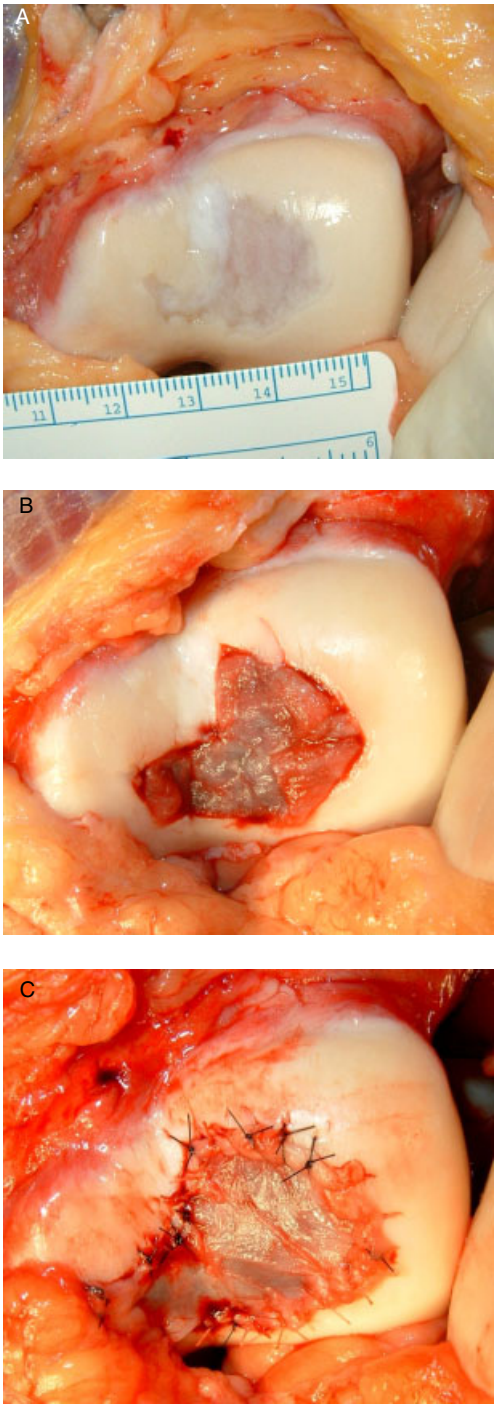

Figure 1. Macroscopic appearance of the patellofemoral joint of the left knee from case 35. (A) Damaged articular cartilage of the femoral patellar groove. (B) Putting cell–gel composite on the defect. (C) Following suturing of autologous synovium in the lesion

osteoarthritis. These 10 knees of 10 patients could not be followed up. We checked the last records of these patients, and found neither tumours nor infections of the operated joints.

We were able to follow up the remaining 31 patients. Among the 45 joints in 41 patients, there were no reports of either tumours or infections between 5 and 137 (mean 75) months after transplantation.

4. Discussion

We consider autologous BMSC transplantation to be a safe procedure because neither tumours nor infections were observed between 5 and 137 (mean 75) months after transplantation among the 45 joints in 41 patients. This study is the first to show the safety of this type of transplantation during a follow-up of nearly 11.5 years. There are reports of BMSC transplantation in immunodeficient mice without tumour generation (Bernardo *et al*, 2007). The observation periods were at mostly 1 year, but some investigators believe this period to be long enough to be relevant to humans when the length of the animal's life is considered. Others considered that these observation periods were not long enough, however, because the transplanted cells were human cells. Thus, we think that the follow-up period of up to 11.5 years of this study is an important feature of the present study.

We could not confirm the present status of 10 of the 41 patients because of death, joint replacement or loss to follow-up. Three patients died (OMMC8, OMMC21 and OMMC22); OMMC8 died 3 months, OMMC21 1 month and OMMC22 23 months after the last visit to our clinic. There were no abnormalities in the transplanted knees in the patients' records at the time of the last visits and the following periods ended at that time.

The possibility that the cells transplanted in joints move and injure other parts of the body remains unresolved. However, this possibility is considered to be low because it has been reported that the MSCs injected into knee joints stayed only in the knee joint (Horie *et al*, 2009). In 2005, spontaneous transformation of human adult stem cells derived from fat tissue from long-term *in vitro* culture (4–5 months) and tumour formation after their *in vivo* transplantation was reported (Rubio *et al*., 2005). This was the first report of the transformation derived from human adult stem cells, and was a surprise for investigators engaged in regenerative medicine. However, there were few reports to confirm the results of this report (Rosland *et al*., 2009). There are many reports concerning animal models, but those on human models were few and information on human models was limited (Ohgushi *et al*., 2005; Toubai *et al*., 2009). As shown in our present results, we could not detect any tumour (transformation). Although long-term culture may show problems of the stem cells, the *ex vivo* manipulation of the human stem cells might be managed safely during the standard expansion period (3–8 weeks).

With regard to infection, before 2000 we cultured cells in a conventional culture room and transplanted them into 25 joints of 24 patients. We used meticulous culture technique and paid special attention to sterilization. No infection was seen in these patients. Currently, there is a consensus that culture for the purpose of regenerative medicine should be performed in a biological clean room (CPC); however, from the fact of our previous (2000) experiences, not only the facility but also the culture technique and caution regarding sterilization are

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especially important to establish safety in using human cell culture.

We transplanted BMSCs into the elbow joints of four children (mean age 13.5 years). Transplantation of BMSCs into young persons is considered problematic because of the length of life remaining to them. We need to observe such patients for longer periods (at present, the longest follow-up period is 6 years). So far, these four patients are satisfied with the outcome of the transplantation.

Autologous BMSC transplantation is a safe procedure and will be widely used around the world.

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