JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **14** (2003) 79–86

The study of metal ion release and cytotoxicity in Co–Cr–Mo and Ti–Al–V alloy in total knee prosthesis – scanning electron microscopic observation

SHIZUKO ICHINOSE*
Instrumental Analysis Research Center, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan

TAKESHI MUNETA, ICHIRO SEKIYA, SOICHIRO ITOH
Section of Orthopaedic Surgery, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan

HIDEKI AOKI
Frontier Research Center, Tokyo Denki University, Ishizaka, Hatoyamamachi, Shikigun, Saitama 305-0394, Japan

MOTOKI TAGAMI
Department of Medicine, Sanraku Hospital, Chiyoda-ku, Tokyo 101-0062, Japan

We surgically retrieved two cobalt(Co)–chromium(Cr)–molybdenum(Mo) and five titanium(Ti)–aluminum(Al)–vanadium(V) alloy knee prostheses from patients because of mechanical failure and pain. We examined the distribution of the small particles which were released from the Co–Cr–Mo and Ti–Al–V alloys using a backscattered scanning electron microscopy (SEM). In addition we analyzed the metals in the artificial knee joints and the tissues adjacent to them using energy dispersive X-ray spectroscopy (EDS).

We demonstrated that a myriad of fine particles, produced by the abrasion of both Co–Cr–Mo and Ti–Al–V alloys, accumulated in the synovial cells. As Co–Cr–Mo alloys disintegrate easily in the cells, Co dissolves from the peripheral areas of them, although Cr remains within the cells. In contrast Ti–Al–V alloys are very stable in the synovial cells.

From these findings we conclude that the Co–Cr–Mo alloys are hazardous to the body as the alloys release Co which enters the body. In contrast the Ti–Al–V alloys are very stable and are patently safer. Artificial joints, however, are still in considerable need of improvement.

Introduction

Artificial joints made from metal and polyethylene are used for functional recovery when patients lose joint function because of osteoarthritis or rheumatoid arthritis. Co–Cr–Mo and Ti–Al–V alloys have recently become alloys of choice in the production of prosthesis [1–6].

There have been several studies concerning the cytotoxicity and dissolution of these alloys [7–13] and the corrosion products released from them [6]. In addition the concentrations of these metals were measured in the blood, urine and tissues of the patients wearing them [2–5]. However, it is uncertain whether these alloys produce corrosion debris as a result of wear and whether the debris is cytotoxic. Thus, it is not absolutely certain which alloys are suitable for implantation.

In this study we surgically retrieved total knee joints made from Co–Cr–Mo alloys or Ti–Al–V alloys. We examined the distribution of the fine particles which were released from these alloys using a backscattered scanning electron microscopy (SEM). We further analyzed the particles in the adjacent tissues of the knee prostheses by energy dispersive X-ray spectroscopy (EDS). By applying these techniques, we tried to clarify the detailed process of the decomposition and corrosion of these alloys *in vivo* and to demonstrate which alloy was appropriate and risk free for the body.

Materials and methods

Cementless artificial knee joints (porous-coated anatomic, PCA type), made from the Co–Cr–Mo alloys, were used in two cases (Table I). In these cases severe knee pain occurred from 5 to 7 years after their operation. As a

*Author to whom all correspondence should be addressed.

TABLE I Patients profile

Case	Diagnosis	Age/Sex	Time to onset (year)	Model of total knee Joint	Origin of wear particles
1	RA	49/F	8	PCA	Co–Cr–Mo alloy, UHMWPE
2	OA	63/F	5	M/GI	Ti–Al–V alloy, UHMWPE
3	OA	75/F	5	M/GI	Ti–Al–V alloy, UHMWPE
4	OA	67/F	7	PCA	Co–Cr–Mo alloy, UHMWPE
5	OA	74/F	3	M/GI	Ti–Al–V alloy, UHMWPE
6	OA	76/F	8	M/GI	Ti–Al–V alloy, UHMWPE
7	OA	73/F	8	M/GI	Ti–Al–V alloy, UHMWPE

RA: rheumatoid arthritis; OA: osteoarthritis; PCA: porous coated anatomic artificial knee joint (Howmedica); M/GI: Miller-Galante Type I artificial knee joint (Zimmer); UHMWPE: ultra high molecular weight polyethylene.

result the patients were unable to walk because of the pain, and the artificial joints had to be removed.

The artificial knee joints (Miller-Galante type I, M/GI type), made from the Ti–Al–V alloys, were used in five cases (Table I). Patients, receiving total knee replacement, suffered from severe knee pain and swelling from 3 to 8 years after the operation. As the pain made walking difficult, these artificial joints were also removed.

The surrounding tissue, including the synovial membrane, was excised from the area around the Co–Cr–Mo and Ti–Al–V alloy joints. They were cut into small pieces ($2 \times 2 \times 1$ mm), fixed in 2.5% glutaraldehyde for 2 hr and dehydrated by graded ethanol. Thereafter the small pieces were dried using a critical point drying apparatus HCP-2 (Hitachi Ltd., Hitachinaka) with liquid CO_2 and then the samples were spatter-coated with osmium using NL-OPC80N (Nippon Laser & Electronics Lab., Nagoya). These synovial tissues were examined by an SEM S-4500 (Hitachi Ltd., Hitachinaka) and a yttrium–aluminum-garnet (YAG) backscattered detector (Hitachi Ltd., Hitachinaka).

In addition, new alloys before operation, metal components and adjacent synovial tissues removed from the patients were analyzed by an energy dispersive X-ray spectroscope EMAX-7000 (Horiba Ltd., Kyoto). Acquisition time of the EDS spectrum was 100 sec at 15 kV of acceleration voltage and 0.1 nA of beam current. The EDS spectrum was quantitatively assessed according to the intensity of each spectrum. The overlapping lines were separated by the overlapped-factor-method and quantitative correction was done after the line separation employing the standardless method [14]. Then a quantitative analysis of the alloy elements was performed and the values of C, O, Na, Ca, P and K were deleted because these elements were consistently detected in every kind of cell. The sum of each value was 100% as it was shown by the wt % of the total volume. EDS spectrum of synovial cells adjacent to the alloy joints was acquired from the areas of $3\,\mu m \times 5\,\mu m$ or $3\,\mu m \times 3\,\mu m$ according to the size of the cells. The spectrum of metal components was analyzed in the areas of $3\,\mu m \times 3\,\mu m$.

Using the methods described above, we examined (1) new Co–Cr–Mo ($n = 6$) or Ti–Al–V ($n = 5$) alloy joints, (2) metal components of Co–Cr–Mo ($n = 6$) or Ti–Al–V ($n = 5$) alloy joints removed from the patients and (3) synovial cells adjacent to the alloy joints ($n = 6$ in Co–Cr–Mo joint; $n = 5$ in Ti–Al–V joint).

The EDS line analysis was done on 50 different synovial cells adjacent to the Co–Cr–Mo (case 1) or Ti–Al–V alloy joints (case 2 and 3). In the analysis of Co, Cr and Mo we used the characteristic X-ray line, that is Cr Kα, Co Kα and Mo Lα, respectively. In addition Ti Kα, Al Kα and V Kα were employed to analyze Ti, Al and V, respectively. As the energy scale was the same throughout the study, the height of the line indicated the value of each element. The statistical significance is determined using Fisher's protected least significance difference following analysis of variance (ANOVA).

Results

Radiograph of the case before revision surgery

Fig. 1 shows the knee joint roentgenogram of case 1. The thick arrow (A) indicates the metal component made

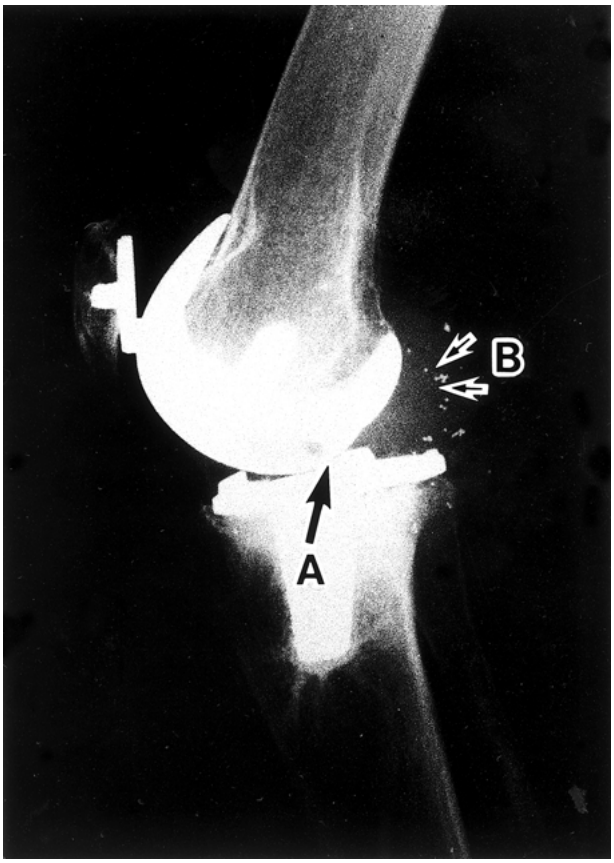

Figure 1 Knee joint roentgenogram of case 1. The thick arrow (A) indicates the metal component of the tibia made from the Co–Cr–Mo alloy. The thin arrows (B) point out the synovial tissues surrounding the artificial knee joint.

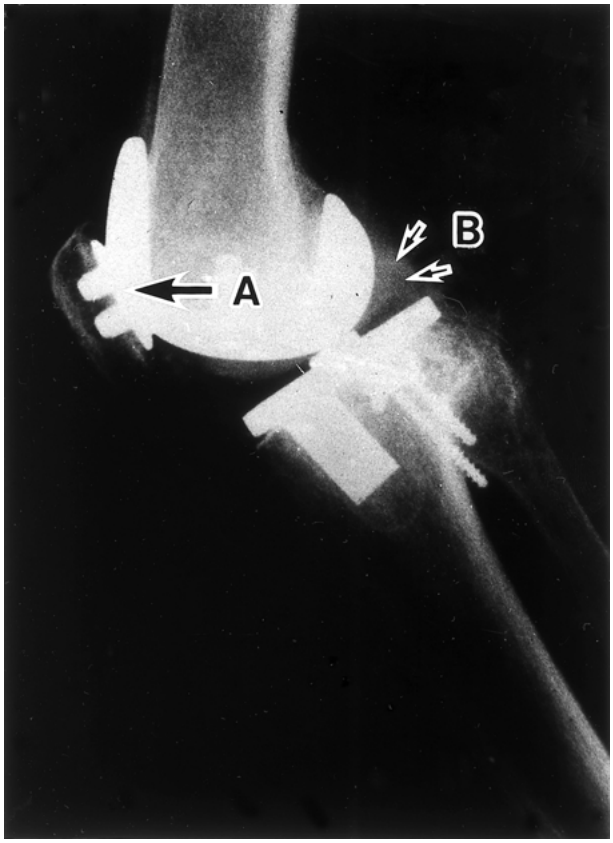

Figure 2 Knee joint roentgenogram of case 2. The thick arrow (A) indicates the metal component of the patella manufactured from the Ti–Al–V alloy. The thin arrows (B) point out the synovial tissue around the artificial knee joint.

from the Co–Cr–Mo alloy for the tibia. The metal disintegrated partially into a myriad of small pieces due to abrasion because the tibial polyethylene surface component deteriorated and the metal of the tibia rubbed directly against the metal component of the thighbone. It was suspected that a large amount of metal debris of Co–Cr–Mo alloy were released into the joint. Fig. 2 is the knee joint roentgenogram in case 2. The thick arrow (A) indicates the metal component of the patella manufactured from the Ti–Al–V alloy. As the surface components, made from polyethylene, were

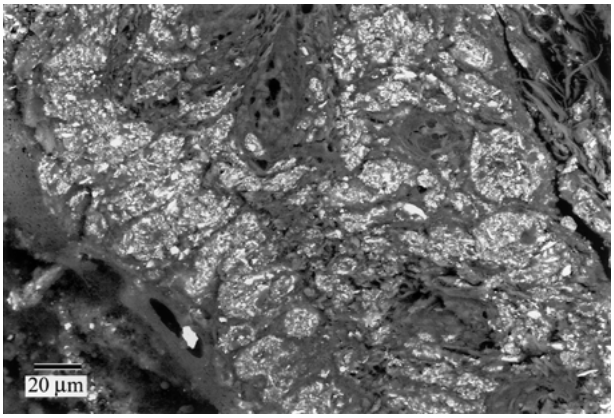

Figure 3 Backscattered SEM image of synovial tissues adjacent to the artificial knee joint made from the Ti–Al–V alloy. Metal debris were present within the synovial tissue.

abraded and became smaller, the metal for the patella directly touched and rubbed the metal thighbone and tibia. Thin arrows (B) show the synovial tissues around the artificial knee joint. It was suspected that a large amount of metal debris of Ti–Al–V alloy wear were released into the joint.

Examinations using an SEM

We examined the synovial tissues adjacent to the artificial knee joints made from the Ti–Al–V alloys using a backscattered SEM (Fig. 3). Fine particles, produced by abrasion of the Ti–Al–V alloys, were flickering white and distributed throughout the synovial tissues (Fig. 3).

We then examined the synovial tissues adjacent to the artificial knee joints made from Ti–Al–V alloys by applying both a secondary SEM and a backscattered SEM. Fig. 4(A) is a photograph of a secondary SEM and Fig. 4(B) is the photograph of a backscattered SEM in the same area. These photographs are of higher magnification than that of Fig. 3. We detected two cells in Fig. 4(A) (indicated by the arrows), and found numerous fine particles within the two cells in Fig. 4(B). These

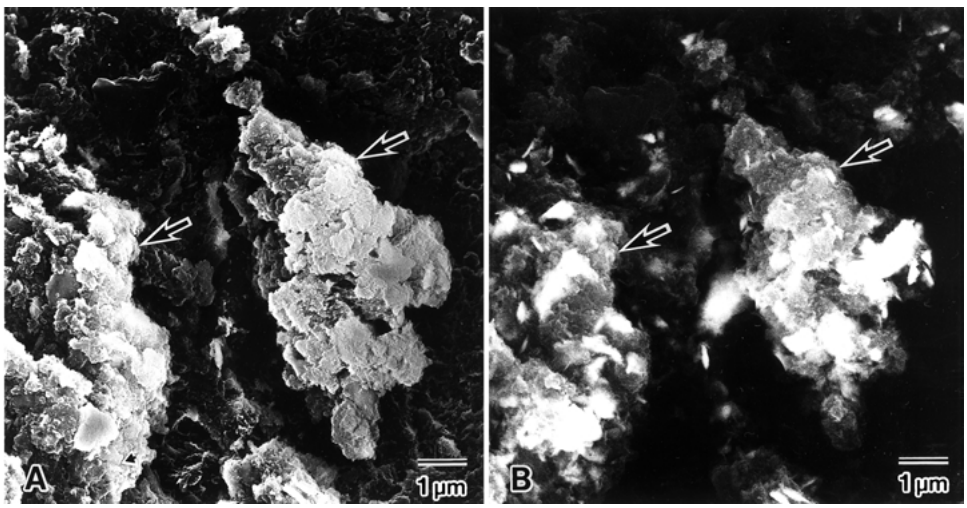

Figure 4 Secondary electron SEM image (A) and backscattered SEM image (B) of the synovial tissue adjacent to the artificial knee joint made from the Ti–Al–V alloy. Two cells (arrows) are detected in Fig. 2(A). Numerous fine particles were observed within the cells in Fig. 2(B).

particles, resulting from the abrasion of the alloys, revealed high-luminosity and a cylindrical structure. Most of them were minute and their width was less than 30 nm, although relatively large deposits were occasionally detected.

Fine particles, made from the Co–Cr–Mo alloys, were also trapped by the cells of the synovial tissues. These findings using an SEM were very similar to those observed in the cases of Ti–Al–V alloys.

Analysis using an EDS

EDS spectrum and quantitative analysis of metal elements in the artificial knee joint and the synovial cell.

We obtained EDS spectrum of the metal components (shown in Fig. 1, thick arrow) and the synovial cells (shown in Fig. 1, thin arrows) by applying EDS.

Fig. 5(A) is EDS spectrum of the Co–Cr–Mo alloy component removed from the patients and Fig. 5(B) is the spectrum of the synovial cells adjacent to the alloys. We prove that the Co–Cr–Mo alloy (Fig. 5(A)) consists of Co, Cr, Mo, Fe, Ni, Al *et al*. In addition the synovial cells adjacent to the alloys contain the alloy elements (Co, Cr and Mo) and the cell components (C, Ca, O, Na and P). Although the heights of Cr and Co are almost the same in the alloy, Co in the synovial cell is very small in height compared with the value of Cr. These results indicate that Co–Cr–Mo alloys disintegrate. Co dissolves and Cr remains within the cell.

We then performed the quantitative analysis using EDS spectrum data. The data on the metal components of case 1 are shown in Table II. The volume ratio of Co, Cr and Mo was 63.29 : 28.01 : 5.12, respectively, on the surface of the metal components removed from patients (Table II, column 2: $n = 6$). As the ratio of Co, Cr and Mo was 61.81 : 28.26 : 6.37, respectively, in the alloys before the operation (Table II, column 1: $n = 6$), the values in the metal components from the patients were the same as in the new alloys.

Table II, column 3 is the data of the synovial cells $(n = 6)$. In the patients with the artificial Co–Cr–Mo alloy joints, the ratio of Co, Cr and Mo was 4.16 : 69.15 : 20.67, respectively. The value of Co was significantly lower $(p < 0.01)$ than those observed in the metal components after the operation and in the

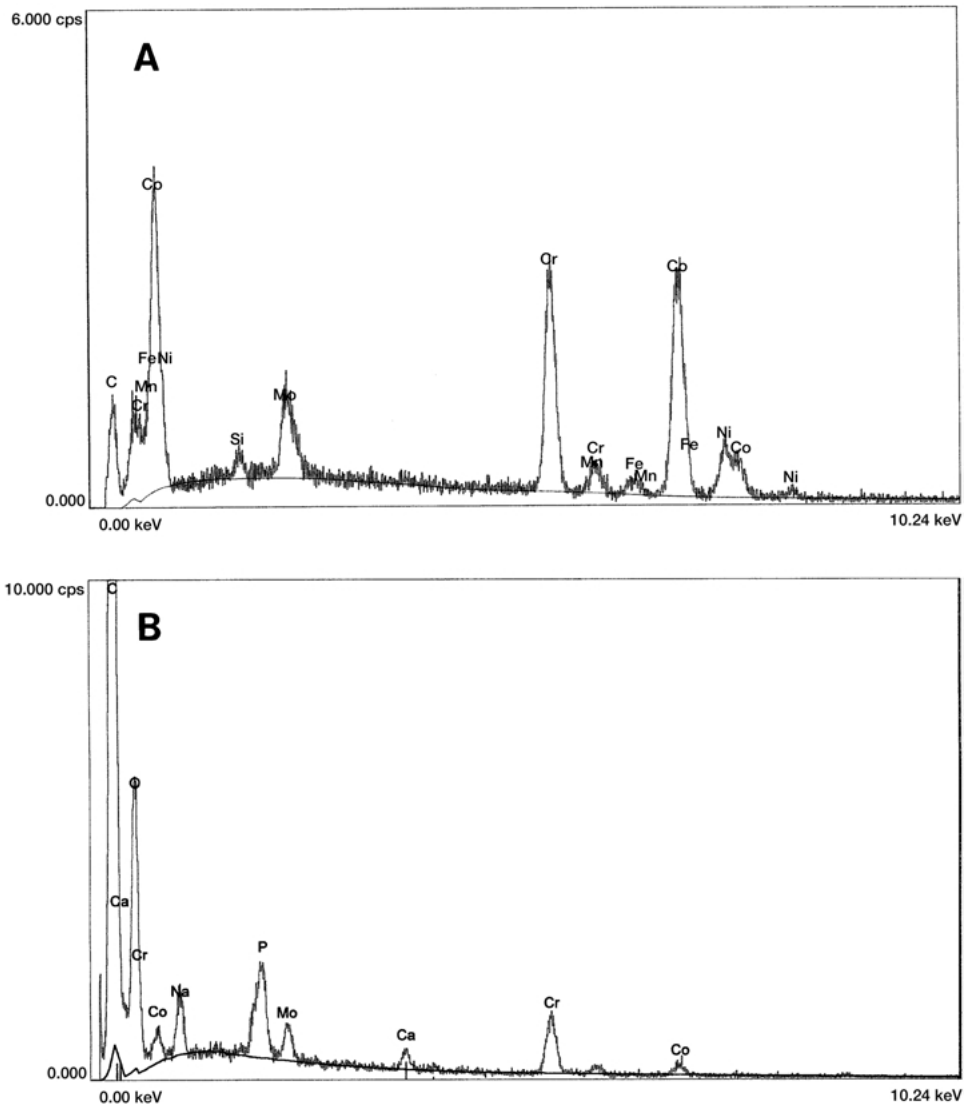

Figure 5 EDS spectrum of the Co–Cr–Mo alloy component removed from the patients (A) and the synovial cells adjacent to the alloys (B). Co–Cr–Mo alloy consist of Si, Cr, Mn, Fe, Co, Ni and Mo. The synovial cells adjacent to the alloys contain, Na, Ca and P which consistently exist within the cells. Co within the synovial cell is extremely small in amount compared with the value of the alloys.

TABLE II Quantitative analysis of the Co–Cr–Mo alloys and the synovial cells adjacent to the alloys from Case 1

	Weight%		
	1	2	3
Si	1.00 ± 0.10	0.94 ± 0.12	0.00 ± 0.00
Cr	28.26 ± 0.33	28.01 ± 0.78	69.15 ± 2.06
Mn	0.79 ± 0.07	0.36 ± 0.15	0.67 ± 0.65
Fe	0.76 ± 0.02	0.29 ± 0.22	2.15 ± 0.50
Co	61.81 ± 0.71	63.29 ± 1.02	4.16 ± 0.95
Ni	1.01 ± 0.47	1.99 ± 0.05	3.22 ± 0.71
Mo	6.37 ± 0.15	5.12 ± 0.13	20.67 ± 1.64

1. The new alloy before the operation. 2. The metal components removed from the patients 3. The synovial cells. The volume ratio of alloy elements were the same as in the alloys removed from the patient (1) and in the new alloy before the operation (2). The volume ratio of Co within the synovial cells (3) was extremely small in amounts compared with the alloys.

new alloys. These data indicate that Co dissolves from the cells and is absorbed by the body when the artificial Co, Cr and Mo alloy joints abrade and are phagocyted by the synovial cells. Case 4 showed a similar tendency.

We examined the EDS spectrum of the metal components (shown in Fig. 2, thick arrow) and the synovial cells (shown in Fig. 2, thin arrows) by applying EDS. Fig. 6(A) is the EDS spectrum of the Ti–Al–V alloy removed from the patients and Fig. 6(B) is the data of the synovial cells adjacent to the alloys. This study demonstrates that the Ti–Al–V alloy consists of Ti, Al and V. The synovial cells adjacent to the alloys contain the alloy elements (Ti, Al and V) as well as the cell components (Na, P, S, K and Fe). The volume ratio of alloy elements were the same as in the alloys and in the synovial cells. These results point out that Ti–Al–V alloys are very stable within the cells.

We then performed the quantitative analysis using the EDS spectrum data. The data of the metal components of case 2 and case 3 are shown in Table III. The volume ratio of Ti, Al and V is 90.12 : 7.22 : 2.66, respectively, on the surface of the metal components removed from the patients (Table III, column 2, $n = 10$ from two cases). Although the ratio of Al is rather low in the metal components after the operation, the values in the component are almost the same as in the new alloy (Table III, column 1, $n = 10$ from two cases). In patients with the artificial knee joints made from the Ti–Al–V

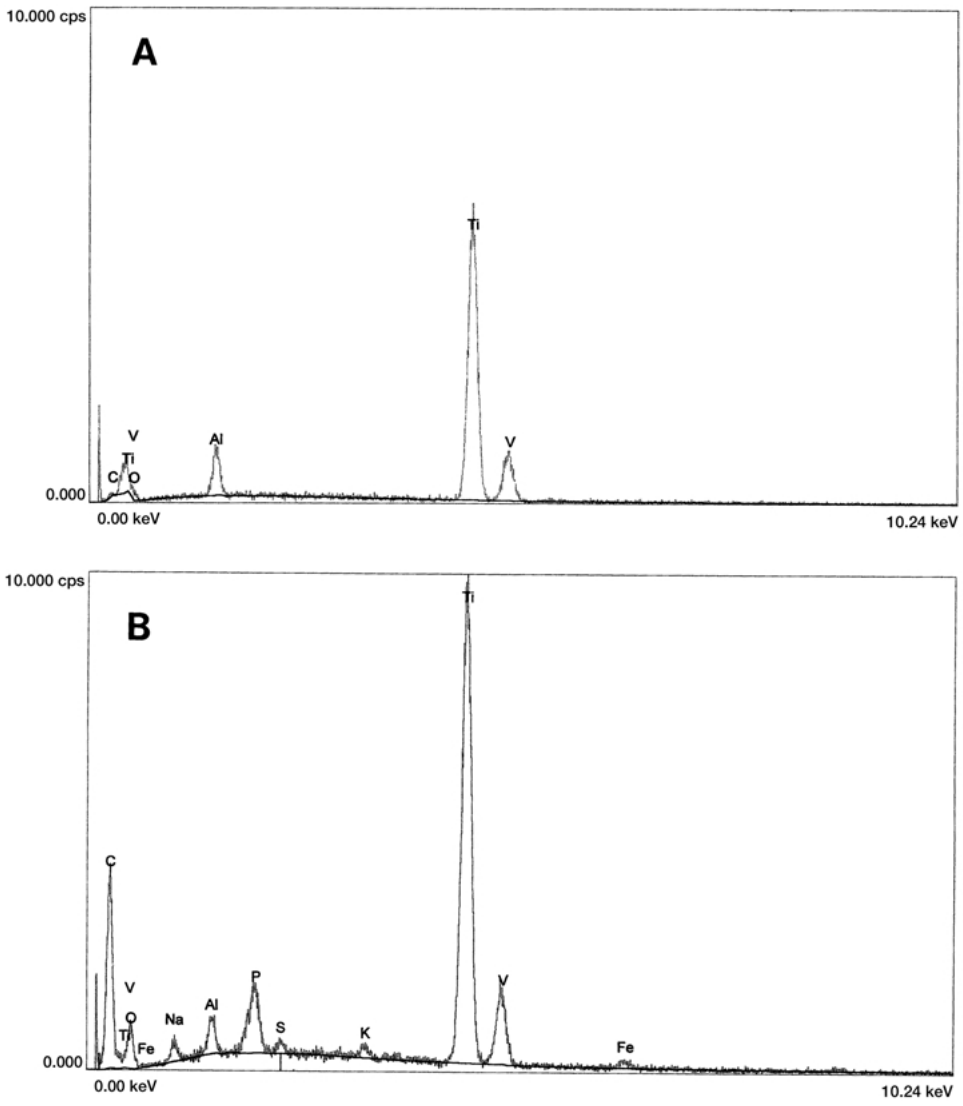

Figure 6 EDS spectrum of the Ti–Al–V alloy removed from the patients (A) and the synovial cells adjacent to the alloys (B). Ti–Al–V alloy consist of Ti, Al and V. The synovial cells next to the alloys consist of alloy elements Na, K, S and P which consistently exist within the cells. The volume ratio of alloy elements were the same as in the alloys and in the synovial cell.

TABLE III Quantitative analysis of the Ti–Al–V alloys and the synovial cells adjacent to the alloys from Case 2 and Case 3

	Weight%		
	1	2	3
Al	6.49 ± 0.56	7.22 ± 0.75	6.36 ± 0.54
Ti	90.13 ± 1.58	90.12 ± 0.25	91.02 ± 0.90
V	3.38 ± 1.75	2.66 ± 0.90	2.62 ± 0.51

1. The new alloy before the operation 2. The metal components removed from the patients 3. The synovial cells. The volume ratio of Ti, Al and V were the same as in the new alloy, in the metal components after the operation and in the synovial cells.

alloys, the volume ratio of Ti, Al and V is 91.02 : 6.36 : 2.62, respectively, in the synovial cells (Table III, column 3, $n = 10$ from two cases). The values of three metals in the synovial cells are similar to those detected in the metal components from the patients and the new alloys. These data confirm that the Ti–Al–V alloy is very stable and does not seep into the body when the artificial joints are abraded by direct contact with metal components. The results in case 5, 6 and 7 were almost similar.

Line analysis of the cell

Figs. 7 and 8 are the results of the line analyses of synovial cells which trapped the fine particles produced by the abrasion of the alloy. The height of the graph from the baseline shows the X-ray strength which corresponds to the relative quantity and distribution of each element within the cell.

Fig. 7 is the result of the line analysis of the synovial cell (indicated by the horizontal line in the top photograph) adjacent to the artificial knee joint manufactured from the Co–Cr–Mo alloy. This analysis demonstrates that the debris from the abrasion of the alloys accumulates in the central area (about 3 μm in width) of the cell.

The height of Co (solid line), Cr (broken line) and Mo (dotted line) indicates the relative quantity of each element in the cell. Although the ratio of Co, Cr and Mo in the alloy is 60 : 33 : 4, respectively, the value of Co, Cr and Mo in the cell is 12 : 20 : 5, respectively. In other words Co markedly decreased in quantity compared with Cr in the cells. The central portion is the highest in the analytical line of Co, although the Cr line shows a box-like contour. These results reveal that Co dissolves from the peripheral area of the cell and is absorbed by the body. As a result Co is detectable in the central area only.

Fig. 8 is the result of the line analysis of the synovial cell (indicated by the horizontal line in the top photograph) next to the Ti–Al–V alloy artificial knee. The analysis demonstrates that two clear peaks of Ti (broken line), Al (dotted line) and V (solid line) are present in the whole area of the cell, the diameter of which is 4 μm. The ratio of Ti, Al and V in the alloy is 86 : 10 : 4, respectively, and the value of Ti, Al and V in the synovial cell is 63 : 15 : 11, respectively. The ratios among the three metals in the alloy are almost the same as those observed in the synovial cells.

These findings indicate that the Ti–Al–V alloy does not dissolve from the cells and is not absorbed by the body, although the alloy disintegrates into fine particles due to abrasion and the abrasive particles are trapped by the synovial cells.

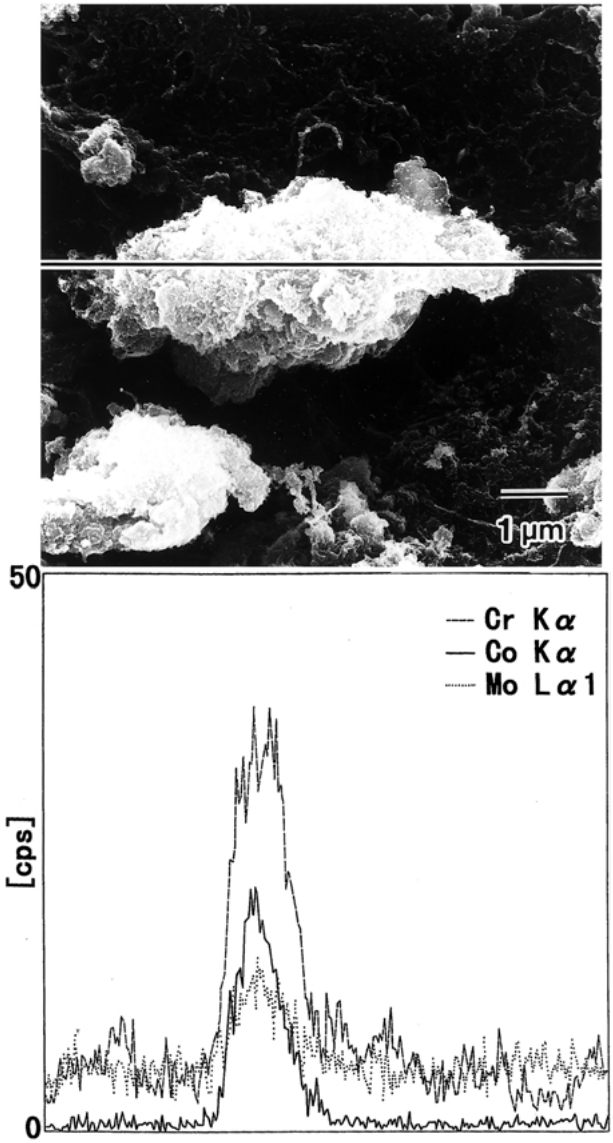

Figure 7 Top: Synovial cell examined by an SEM. Bottom: Linear analysis of the area indicated by the horizontal line in the top photograph using energy dispersive X-ray spectroscopy. The height of Co (solid line), Cr (broken line) and Mo (dotted line) indicates the distribution and the relative quantity of each element in the synovial cell.

Discussion

Many researches have examined wear particles abraded from artificial knee joints manufactured from Co–Cr–Mo and Ti–Al–V alloy postoperatively. However, it is still unclear which alloy is physiologically ideal for the manufacture of artificial joints. Evans *et al*. [7] pointed out that toxicity was strongest in the smallest abrasive particles released from the Co–Cr–Mo alloy after they examined the cytotoxicity of the particles in different diameters using the Co–Cr–Mo and Ti–Al–V alloys. Haynes *et al*. [8] revealed that toxicity was strong in the Co–Cr–Mo alloy when the fine particles from the alloys were of equal size. Maloney *et al*. [9] studied the toxicity of Ti, Ti–Al alloy, Co and Cr of identical sizes and reported that the cytotoxicity was highest in Co. It was

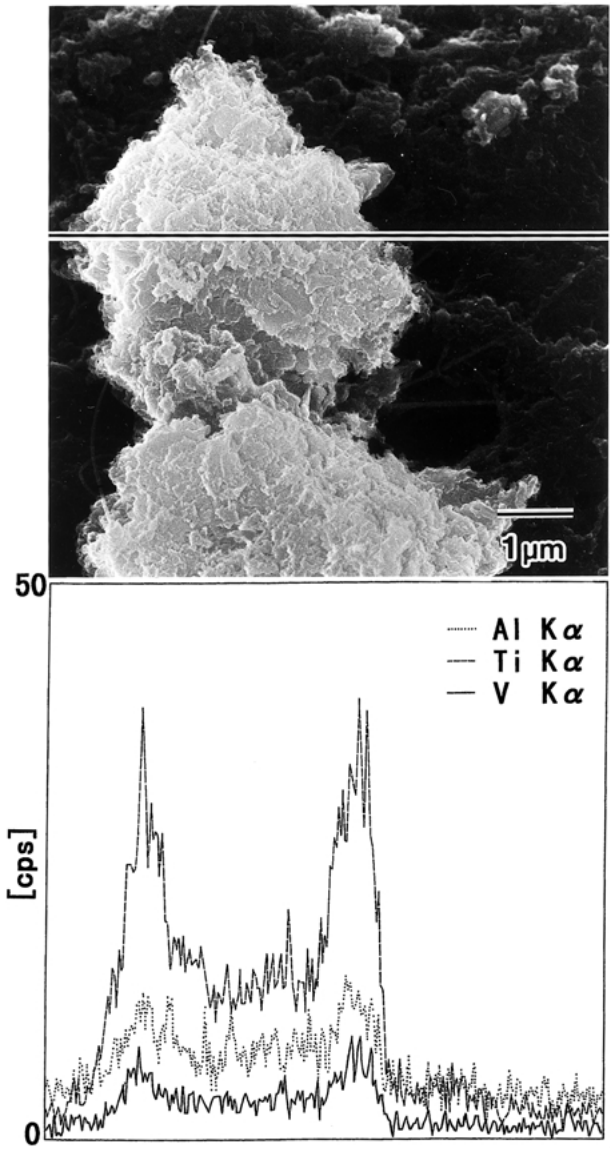

Figure 8 Top: Synovial cell examined by an SEM. Bottom: Linear analysis of the area indicated by the horizontal line in the top photograph using energy dispersive X-ray spectroscopy. The height of Ti (broken line), Al (dotted line) and V (solid line) indicates the distribution and the relative quantity of each element in the synovial cell.

also evident that Co and V were higher in cytotoxicity than that found in Ni, Cr, Mo, Ti and Al [10], and that Co and Cu readily dissolved into a protein solution compared with Al, Cr, Mo, Ni and Ti [11].

There are several studies reporting the effects of these metals on the human body. Dobbs *et al*. [2] proved that Co concentration was high in the blood and urine of patients wearing artificial Co–Cr–Mo alloy joints, and that Cr was detected in the synovial tissues. Black *et al*. [3] demonstrated that the concentration of Co gradually decreased when the Co–Cr–Mo alloy joints were removed, although the value of Cr increased in the blood postoperatively. In addition, Woodman *et al*. [15] indicated that Co dissolved into the fluid although Cr was present in the tissues around the alloy when the Co–Cr–Mo alloy was implanted. Furthermore, two groups examined the Ti–Al–V alloy *in vivo*. Jacobs *et al*. [4] proved that the concentration of Ti was high in the blood of patients who underwent artificial joint replacement. Agins *et al*. [5] pointed out that the concentration of Ti, Al and V increased in the synovial tissues, although Ti concentration was the lowest among the three elements. V is highly cytotoxic [10, 16, 17] and Al is a cause of Alzheimer disease [17, 18]. If these elements release, it is hazardous to the body.

In this study we examined the distribution and size of fine particles which were released from the Co–Cr–Mo and Ti–Al–V alloys using a backscattered SEM. We further analyzed the particles by EDS. We demonstrated that numerous fine particles were found within the cells adjacent to the artificial joints using an SEM. These wear particles were produced by the abrasion of the alloys and showed high-Iuminosity and a cylindrical structure.

The line analyses by EDS revealed that the ratio of Co, Cr and Mo was 60 : 33 : 4 in the alloys, respectively. In contrast the value of Co in the synovial cells was markedly low compared with that of Cr. In addition this study showed that most of Co was present in the central area of the cells, although Cr was found in the wider areas of the cells. In addition our quantitative analyses demonstrated that the volume ratio of Co, Cr and Mo in the metal components removed from the patients was the same as in the new alloy before the operation, and that the value of Co in the synovial cells was significantly lower than those observed in the metal components after the operation and in the new alloys.

Our line analysis then verified that two peaks of Ti, Al and V were clearly present throughout the cells next to the Ti–Al–V alloy joints. The ratios among the three metals in the synovial cells were almost the same as those observed in the alloy. Our quantitative analyses also revealed that the volume ratio of Ti, Al and V in the synovial cells was the same as those detected in the alloys removed from the patients and in the new alloy before the operation. These data demonstrate that the Ti–Al–V alloy is stable and does not dissolve when the artificial joints are abraded by direct contact with metal components and the fine abrasive particles are phagocyted by the synovial cells. Ti concentration in the blood and synovial tissues was studied in patients who underwent artificial joint replacement [4, 5]. These studies were performed using atomic absorption spectrophotometry which is very sensitive to the changes in concentration. In this study we did not find any release of Ti from wear particles within the synovial cells using EDS examination. Therefore, we conclude that the increase in Ti concentration is very small in amount and negligible from the clinical point of view in joint replacement.

Recently a better Co–Cr–Mo alloy which has almost eliminated Ni containment was developed and used clinically [19], because Ni causes metal sensitivity [20, 21]. As Co and Cr were also reported to show metal sensitivity [20, 21], these metals should be used carefully for biomaterials. With regard to Ti–Al–V alloy, a Ti-6 Al-7 Nb alloy has been developed [22] to replace V as V is highly cytotoxic [10, 16, 17]. It is evident that Ti–Al–V alloys produce large amounts of wear particles compared with those in Co–Cr–Mo alloys when direct contact against metal components occurs. Consequently rapid abrasion of Ti–Al–V alloys has become an important problem in joint replacement [5]. Although a

Ti–Zr based alloy has been proved to be safe and shows good mechanical strength [23], alloys containing Ti need urgent improvements including the surface component made from polyethylene.

From these findings we conclude that the Co–Cr–Mo alloys are unsafe because they deteriorate markedly and release Co which is deleterious to the body. In contrast the Ti–Al–V alloys are very stable, do not generate seepage and are more compatible to the body. Artificial joints, however, are still greatly in need of improvement.

Acknowledgments

The authors thank Miss Miyoko Ojima for technical assistance.

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Received 20 April 2001
and accepted 12 June 2002