Evaluation of magnetic behaviour and *in vitro* biocompatibility of ferritic PM2000 alloy

M.S. FLORES[1], G. CIAPETTI[2], J.L. GONZÁLEZ-CARRASCO[3]*, M.A. MONTEALEGRE[3], M. MULTIGNER[1], S. PAGANI[2], AND G. RIVERO[1]
[1]*Instituto de Magnetismo Aplicado. Laboratorio ''Salvador Velayos'', RENFE-UCM-CSIC, Madrid, Spain*
[2]*Istituti Ortopedici Rizzoli, Laboratorio di Fisiopatologia degli Impianti Ortopedici, Bologna, Italy*
[3]*Centro Nacional de Investigaciones Metalúrgicas, CENIM-CSIC, Madrid, Spain*
E-mail: jlg@cenim.csic.es

PM2000 is a ferritic alloy obtained by powder metallurgy and is being investigated for potential applications as a biomaterial. This work aimed to assess the biological compatibility and to determine the influence of the processing route and further recrystallisation treatment on the magnetic behaviour. The magnetic behaviour has been analysed as a function of the hysteresis loop obtained by using an inductive method. The biocompatibility has been tested using human osteoblast-like cells seeded onto discs of PM2000. The ability of cells, on its surface, to attach, grow, and produce alkaline phosphatase (ALP) was determined. It is shown that PM2000 is a soft magnetic material irrespective of its material condition, its remanent magnetisation being very low (up to about 3% for the recrystallised swaged material). Fields close to 200 Oe are required to saturate the material. The saturation magnetisation is about 135 emu g^{-1}. *In vitro* tests indicate that cells are able to attach and grow onto its surface, and produce ALP, a specific marker of cells with bone-forming activity. In this respect, PM2000 holds promise as a suitable substrate for bone integration. These properties could make PM2000 a useful candidate for the preparation of medical devices where biocompatible and soft magnetic materials are sought. Applications for dental magnetic attachments could be envisaged.

1. Introduction

Magnetic attachments that are investigated for dental appliances consists of a magnet combined with a yoke cap and its keeper [1, 2]. Not only excellent magnets but also soft magnetic stainless steel with high corrosion resistance should be developed for such magnetic attachments [3]. In high-purity ferritic stainless steels, chromium, added to improve the corrosion resistance, decreases the saturation magnetisation. According to Takada and Okuno [4], chromium contents below 17% might limit their use in dental magnetic attachments.

PM2000 (a trademark of Plansee) is an oxide dispersion strengthened (ODS) Fe–20Cr–5Al alloy that combines excellent oxidation and corrosion resistance with good temperature strength; thus, it is widely used for high-temperature applications. The high-temperature strength results from the presence of fine, stable, and uniformly distributed particles of yttria. The superior oxidation resistance is due to the formation, at elevated temperatures, of a fine, dense, and tightly adherent α-alumina scale. Recently, it has been reported [5] that the alloy shows excellent corrosion resistance in simulated human fluids (Hank's solution), which is relevant for their potential applications in the biomaterials field. A deeper investigation of this alloy has been performed as part of the European Research Project ALUSI aimed to develop alumina forming alloys as new biomaterials for surgical implants [6].

The alloy is obtained by a powder metallurgical (PM) route consisting of mechanical alloying of elemental powders in a high-energy ball mill. The consolidation of the powders to obtain a fully dense material is performed by different thermomechanical processes. Hot isostatic pressing (HIP) is used to obtain fully dense billets as a perform to all the other forming methods (rolling, extrusion, forging, etc.), each stage of the consolidation process being interdependent. Variation of processing parameters may gives rise to significant changes in the microstructure (grain size, texture), which should yield different mechanical properties as observed for a similar ODS alloy [7]. It is well known that magnetic properties can be sensitive to the alloy microstructure, impurity elements, and imperfections, whatever their nature. For

*Author to whom all correspondence should be addressed.

instance, small differences in the coercitive force and residual magnetisation can be observed [8] for a similar ODS alloy processed by hot rolling before and after annealing at 1100 °C.

This work is, therefore, aimed to correlate the magnetic behaviour of PM2000 with the microstructure after different processing routes (hot rolling, hot extrusion, swaging, and plate rolling) including further recrystallisation treatments at high temperature. The biocompatibility of the alloy has been tested *in vitro*, using human osteoblast-like cells seeded onto discs. At three and seven days from seeding, the ability of cells, on the surface of the solid samples, to attach, grow, and produce alkaline phosphatase (ALP) was determined.

2. Materials and methods

2.1. Material characterisation

PM2000, whose nominal composition (mass%) is 20 Cr–5.5 Al–0.5 Ti–0.5 Y2O3 balance Fe, was supplied by Plansee Gmbh (Lechbruck, Germany). The alloy was manufactured by a complex PM route involving mechanical alloying to form a fine dispersion of yttrium oxide particles, canning, and compactation at high temperature by different routes. HIP is a process in which the mechanically alloyed powder is subjected to equal pressure from all directions at a temperature high enough for sintering to take place. HIP consolidation is used to obtain fully dense billets as a perform to all the other forming methods. To obtain the rolled and extruded products, HIP densified round material was reduced in thickness to the desired dimensions. To the hot rolled bar described above a step of swaging was introduced to change the texture of the material. Plate rolling was done under the same conditions as bar rolling but in a 2D process. Recrystallisation treatment consists of isothermal annealing above 1100 °C for more than 10 min.

Samples for microstructural characterisation of the substrate were prepared from both as-received and heat-treated materials by conventional metallographic techniques. Examination included optical microscopy and scanning electron microscopy (SEM).

Vickers hardness was determined using loads of 1 kg for 15 s. A minimum of 10 indentations was obtained for each material condition.

Texture measurements were carried out by means of the Shultz reflection method, using a Siemens diffractometer furnished with a D5000 goniometer and a close Eulerian cradle. The X-radiation was α-filtered Cu Kα. The specimens were discs of 2 mm thickness with the disc plane perpendicular to the longitudinal bar direction. Interpretation of the texture was based on the (1 1 0), (2 0 0), and (2 1 1) pole figures and from the orientation distribution function (ODF) calculated by means of a series expansion method.

The magnetic behaviour has been analysed as a function of the hysteresis loop obtained by using an inductive method [9]. For these measurements, rods of about 6 mm diameter and 10 cm length were removed from the original bar. Hysteresis loops, with a maximum applied field of 400 Oe, were recorded along the longitudinal bar direction before and after the recrystallisation treatment.

2.2. *In vitro* cell testing

Discs of PM2000 were sized to fit to the bottom of a 12-well cell culture plate (∅ 20.5 mm). Cells seeded onto tissue culture polystyrene (TCPS) provided the controls (crl).

2.2.1. Cells

Saos-2 are osteoblast-like cells from human osteogenic sarcoma (ATCC, HTB 85), which retain many characteristics of osteoblasts, including the release of alkaline phosphatase and the ability to form mineralised nodules *in vitro* when fed with the appropriate additives [10]. The culture medium was a modification of Eagle' medium (α-MEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin. The cells were maintained in continuous culture at 37 °C in an atmosphere of 95% air/5% CO_2 and 95% humidity.

2.2.2. Cell seeding

The PM2000 discs were pre-wetted with α-MEM for 30 min. under laminar airflow, then placed at the bottom of the 12-well culture plates. Saos-2 cells were seeded 1×10^5 per sample in the complete culture medium. Following incubation at 37 °C, the cells were assayed for viability, proliferation, and ALP production after three and seven days. Their morphology was observed using a fluorescent light microscope following staining with acridine orange.

2.2.3. Methods

Cell viability/metabolism on the biomaterial surfaces was measured using Alamar blue reagent (Serotec, Oxford, UK), which measures the redox reactions in viable cells. The dye (10% in medium v/v) was added to the wells at 37 °C for 4 h. The colour change was read using a Cytofluor 2350 (Millipore Corporation) reader at 530ex–590em nm wave length. The results were expressed as percentage of control cells.

The number of cells was determined by visual counting with an hemocytometer. The cells were detached from the samples or TCPS wells using trypsin/EDTA 0.25% for 5 min at 37 °C. After trypsin removal and resuspension in culture medium, the cells were counted using a light microscope and the results expressed as percentage of control cells.

The release of ALP was measured following incubation of 100 µl of the cell supernatants with 100 µl of *p*-nitrophenylphosphate solution (16 mM, Sigma) in glycine buffer for 15 min. at 37 °C. The production of *p*-nitrophenol in the presence of ALP was measured by monitoring light absorbance by the solution at 405 nm, using a reader for microplates (Spectra III, Tecan, Austria). Results were divided by the number of cells (mmol per cell).

Acridine orange staining was used to observe the morphology of the cells grown onto PM2000. The dye is able to stain DNA and RNA green or light red, respectively, and can be observed by fluorescence microscopy. After fixation with 3.7% formaldehyde in PBS for 10 min, the cells are permeabilised with

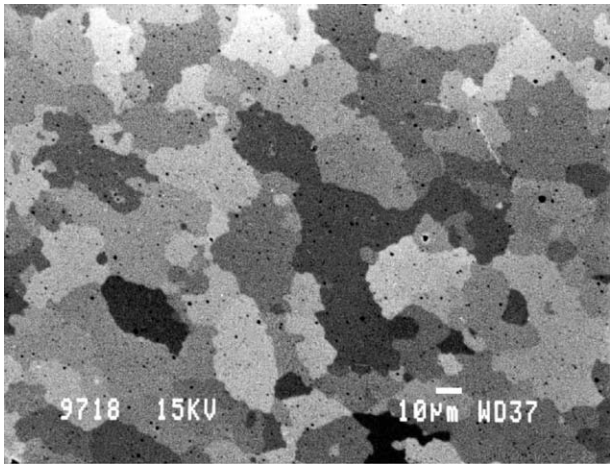

Figure 1 Cross-sectional view (BEI image) of the recrystallised swaged material.

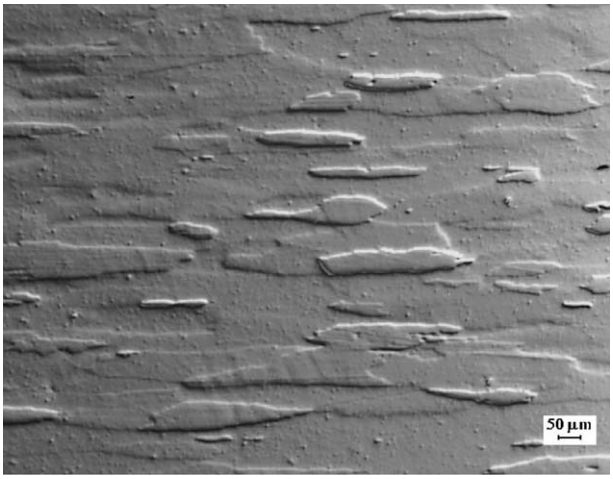

Figure 2 Longitudinal sectional view (optical micrograph) of the recrystallised swaged material.

0.1% Triton X-100 in buffer. Then, acridine orange (Molecular Probes, Eugene, OR) 6 µg/ml in EDTA-buffer is added to the cells at room temperature for 10 min. The cell layer is washed with PBS, covered with a 1 : 1 solution of glycerol and PBS to prevent photobleaching, and observed using a Nikon microscope with an epifluorescence attachment.

3. Results and discussion

3.1. Microstructure

PM2000 in the as-received condition has a structure of very fine grains, irrespective of the processing route. In the case of non-isostatically deformed material SEM examination reveals the existence of grains, about 0.5 µm in diameter, elongated in the longitudinal rod direction. Recrystallisation results in a moderated grain growth in the case of the hipped, rolled, plate rolled, and extruded materials. In the case of the swaged material, however, higher grain growth is observed. It is interesting to note that the average grain size changes from about 100 µm in diameter, in the outer part of the rod, to about 20 µm in the inner part of the rod (Fig. 1). Grains in the non-isostatically deformed materials continue to be elongated in the longitudinal bar direction (Fig. 2).

A clear texture, that is, preferred orientation of grains, has been detected for the non-isostatically deformed materials with and without recrystallisation treatment. The main components of the texture are given in Table I. A ⟨1 1 0⟩ fibre texture is clearly identified in the as-received condition, irrespective of the type of hot deformation. Recrystallisation treatment gives rise to texture change, which is more evident in the case of the swaged material where ⟨1 1 1⟩ becomes the more intense texture component.

Table II shows the hardness values for the different material states. Differences in hardness between materials in the as-received condition are related to small differences in the grain size resulting from the different levels of deformation during processing. As can be seen, annealing of the materials gives rise to a softening that is consistent with the grain growth and annihilation of microstructural defects. It is interesting to note that the absolute hardness decrease is higher for the swaged material, which is consistent with the more severe deformation during this processing. The higher the imposed deformation the larger the stored energy, which is the driving force for the recrystallisation of the material.

TABLE I Texture in the as-received condition and after recrystallisation

Material state	As-received	Recrystallised
Swaged	⟨1 1 0⟩	⟨1 1 1⟩
Rolled	⟨1 1 0⟩	⟨1 1 0⟩; ⟨1 0 0⟩
Extruded	⟨1 1 0⟩	⟨1 1 0⟩
Hipped*	—	—
Plate rolled	⟨1 1 0⟩	⟨1 1 0⟩

*Due to homogeneous ''deformation'' during compactation the hipped material is texture-less.

3.2. Magnetic characterisation

The hysteretic behaviour of the different materials was investigated by analysing the hysteresis loops obtained along the longitudinal rod direction. Fields close to 200 Oe are required to saturate the material, irrespective of the material state. Table III summarises the hysteretic parameters coercitive field (H_c), saturation magnetisation, and remanent magnetisation (M_r), that is, the magnetisation present in the material after removing the applied field for the investigated materials in the as-received and recrystallised conditions. In the as-received condition, there is not an apparent difference between the hysteresis loops, except for the swaged material, for which an increase in H_c and M_r values was found. Recrystallisation treatment of the investigated materials results in a decrease of the coercivity field and the

TABLE II Hardness values for the investigated materials

Material state	As-received	Recrystallised	Hardness decrease
Swaged	394 ± 14	296 ± 6	98
Rolled	331 ± 2	257 ± 6	74
Extruded	356 ± 3	298 ± 10	58
Hipped	343 ± 4	284 ± 4	59
Plate rolled	400 ± 2	342 ± 3	58

TABLE III Hysteretic parameters of the specimens before and after the recrystallisation treatment

Material state	Coercitive field (Oe)		Saturation magnetisation (emu g^{-1})		Residual magnetisation (emu g^{-1})	
	As-received	Recrystallised	As-received	Recrystallised	As-received	Recrystallised
Swaged	18	3	132	138	34	6
Rolled	10	9	134	139	19	14
Extruded	9	8	127	130	17	15
Hipped	5	5	138	137	8	9
Plate rolled	8	8	123	126	20	15

remanent magnetisation. This situation is illustrated in Fig. 3 for the swaged material. This effect has two well-known causes: (i) the enlargement of the grain size and (ii) the relaxation of the internal stresses [11].

The higher values of coercitive field and remanent magnetisation for the as-received swaged material implies the existence of a higher level of internal stresses induced by the more severe plastic deformation during processing. This feature should have increased the driving force for recrystallisation during high-temperature annealing, which is consistent with its highest absolute hardness decrease value. Further evidence of the influence of internal stresses developed during deformation was provided by analysing the hysteretic behaviour of the mechanically alloyed powders used for the consolidation stage. The hysteresis loops are shown in Fig. 4 for powders having average particle sizes of 20 and 100 μm. As can be seen, the coercitive field is higher than for the consolidated material, which is consistent with the fact that in this state the material exhibits the highest level of internal stresses since the material was highly deformed during mechanical alloying at ambient temperature. Further thermomechanical processing at high temperature, used to obtain a dense material, allows a homogenisation of the microstructure, which is accompanied with the annihilation of a large number of defects and decrease of internal residual stresses.

Direct comparison with magnetic properties of other soft ferromagnetic Fe-based alloys was not possible since specimens of the same geometry were not available. Nevertheless, it worth to remark that saturation magnetisation is closely related to the Fe percentage of the alloy. It is well known that an increase of the iron content will increase the saturation magnetisation values, which will result in an increase in the attractive force to the magnet. The limiting factor is that an increase of the Fe content leads to a decrease of the *in vitro* corrosion resistance. From this point of view, PM2000 has an iron content of about 69 at. %, which is higher than that of 64% for a Fe–Pt alloy selected to make attachment keepers [12]. The higher iron content of PM2000 does not compromise the good corrosion behaviour, specially when coated with alumina by thermal oxidation for which a superior *in vitro* corrosion has been reported [13].

3.3. Cell compatibility

After three days the Saos-2 cells show a good viability/metabolic activity, which is confirmed after seven days (Fig. 5); the cells are able to grow onto the PM2000 surface and the amount of ALP released is quite the same as for control cells (Figs. 6 and 7).

The examination by fluorescence microscopy confirmed the adhesion and spreading of cells onto PM2000, which retained their typical morphology for three days (Fig. 8). After seven days the cells formed a dense monolayer so that the surface area was totally covered (Fig. 9).

Additional proof of the lack of toxicity of PM2000 was previously reported [14] after testing the reaction of cells

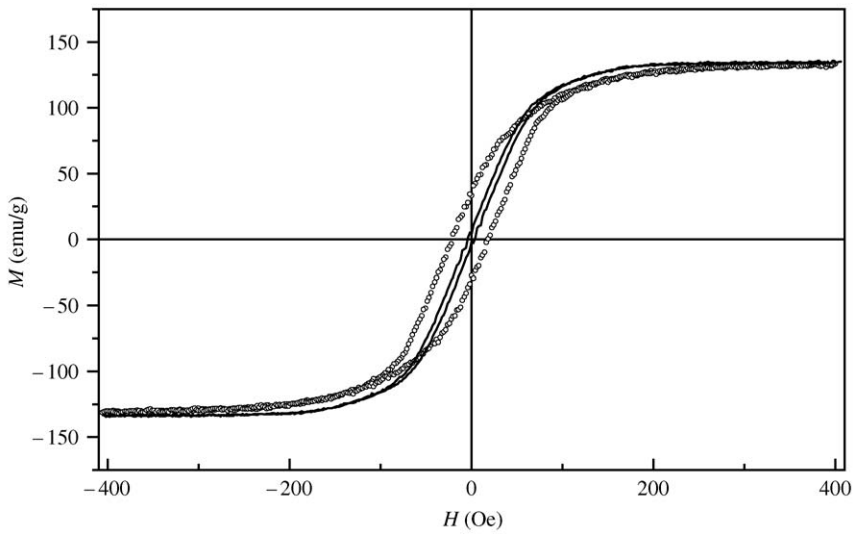

Figure 3 Hysteresis loops corresponding to the as-received (o) and recrystallised (−) samples of swaged material.

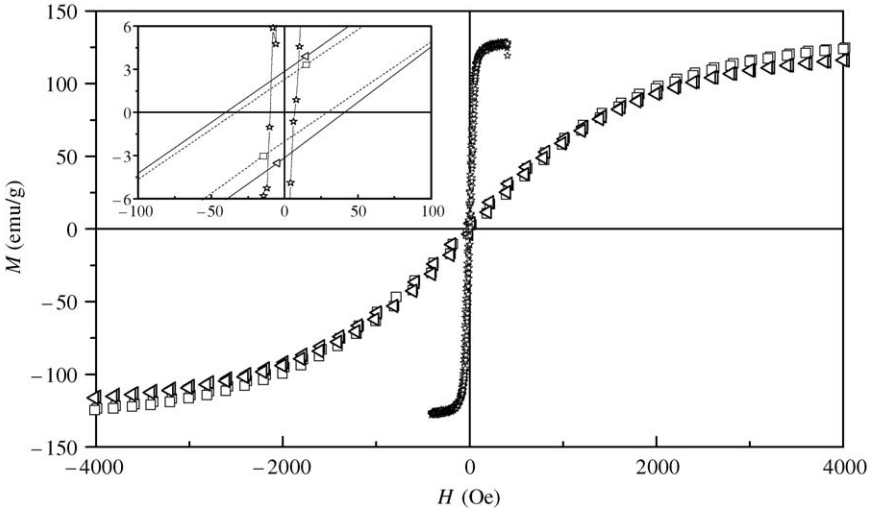

Figure 4 Hysteresis loops corresponding to mechanically alloyed powder of PM2000 with average powder particle size of 100 μm (□) and 20μm (△), and PM2000 extruded (*). Inset at a higher scale showing differences in the coercivity field.

to different amounts (up to 1 mg/ml) of fine metal powders ($< 20\,\mu m$). Testing with particles seems to be a sensitive and reproducible test of biocompatibility. Results on $Saos_2$ and J774A.1 cells, that is, an osteoblast-like and a macrophage cell line, respectively, were reported and compared with the behaviour of powders of the commercial Ti–6Al–4V alloy. The cells were challenged *in vitro* with micrometer-sized particles of about 18 μm for 24 and 48 h. PM2000 was unable to alter cell viability and growth in the short term, even when massive phagocytosis occurs (Fig. 10). The final step involving the use of primary cells from human bone [6, 15] has shown that biocompatibility of PM2000 approaches the behaviour of biomaterials well known for their biological acceptance for example, during. In conclusion, PM2000 seems to be promising with regard to interactions with cells involved in tissue reaction.

4. Concluding remarks

From the analysis of these results it follows that PM2000 is a soft magnetic material irrespective of the material condition. In particular, it is important to note that the remanent magnetisation is very low, less than 10% the saturation magnetisation, being the lowest (about 3%) for the recrystallised swaged material. As far as the biocompatibility is concerned, PM2000 is not cytotoxic, even when tested as fine particles. Experiments with solid samples in the present work has shown that bone cells are able to attach and grow onto its surface, and produce ALP, a specific marker of cells with bone-forming activity. Osteoblasts are the main active cells at the implant interface; therefore, their adhesion and ability to grow and function is the very first step in bone/implant interaction [16]. In this respect, PM2000 holds promise to be a suitable substrate for bone integration. Evidence of the good corrosion behaviour while immersed in simulated human fluids (Hank's solution) has been reported for this [5, 13] and similar [17, 18] ODS Fe–Cr–Al alloys, which is relevant for their potential applications in the biomaterials field.

These properties could make PM2000 a useful candidate for the preparation of medical devices where biocompatible and soft magnetic materials are sought. For instance, this material is suitable as a component of an artificial sphincter, which is being developed by some of the present authors (M.F.S., M.M., and G.R.) within a National Research Project, funded by INDAS (Spain). Applications ranging from the simple use for retention devices, assistors, bone distraction, to maxillofacial applications, orthopaedics, and fracture healing could be envisaged. The ferromagnetic character will

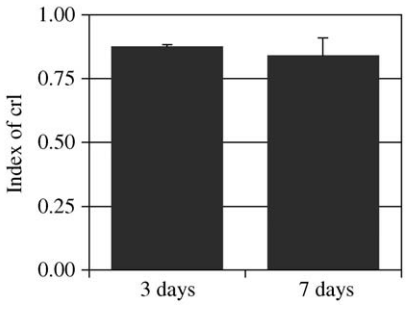

Figure 5 Viability/metabolic activity of Saos-2 cells onto PM2000 (Alamar blue test).

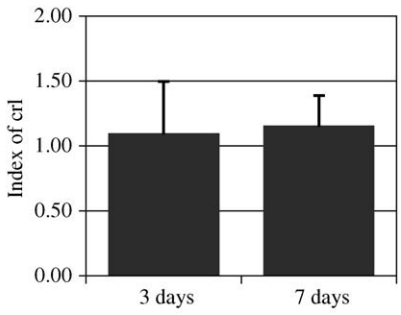

Figure 6 Number of Saos-2 cells grown onto PM2000 (cell count).

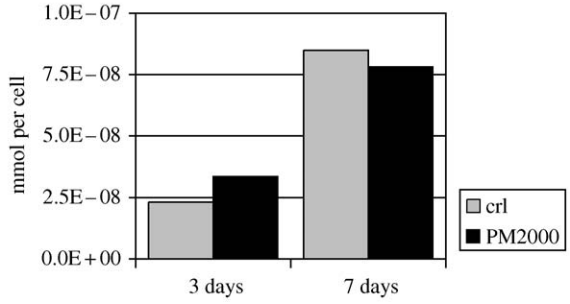

Figure 7 Release of alkaline phosphatase from Saos-2 cells onto PM2000.

obviously prohibit the use of medical control techniques based on strong magnetic fields [19, 20], primarily because of the risks associated with their movement or dislodgment, a restriction affecting also to patients wearing for, instance, cardiac pacemakers, electrical implants, prosthetic cardiac valves, aneurysm clips, or simply rejected by discomfort.

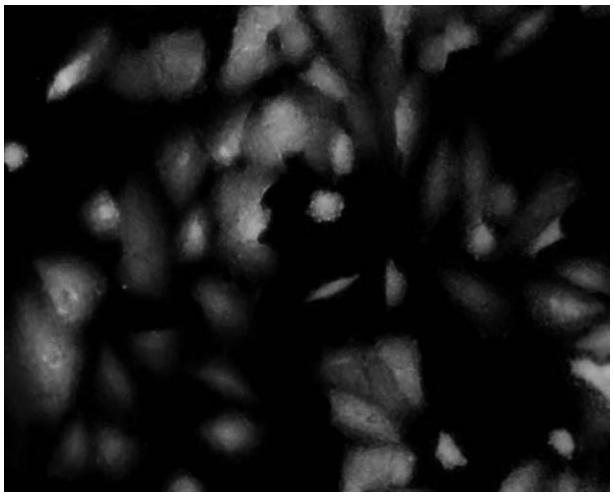

Figure 8 Saos-2 cells after 3 days onto PM2000 (AO, 20 ×).

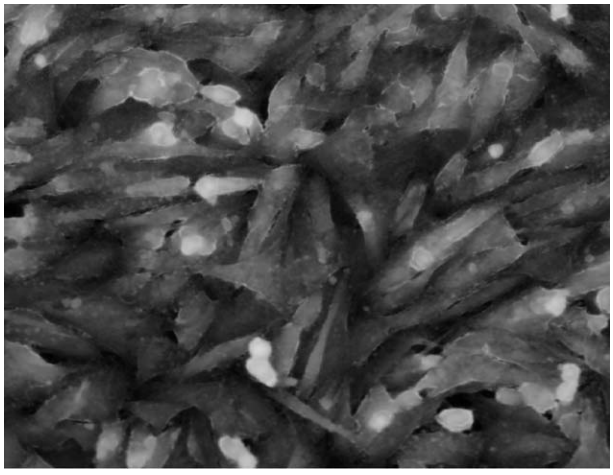

Figure 9 Saos-2 cells after 7 days onto PM2000 (AO, 20 ×).

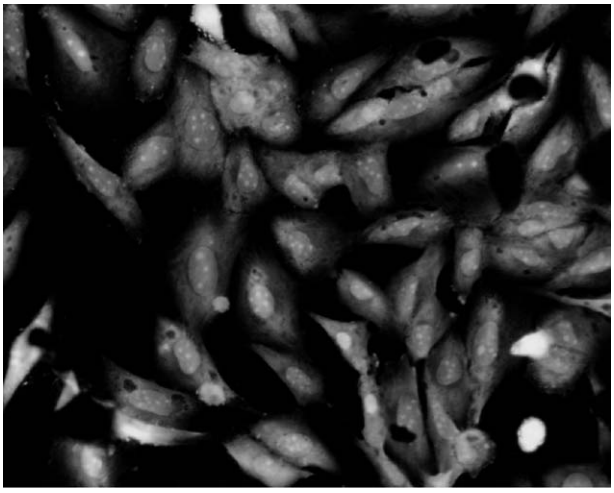

Figure 10 Saos-2 cells after 7 days with PM2000 particles (black spots onto cells) (AO, × 20).

Acknowledgments

The authors would like to thank Plansee Gmbh (Lechbruck, Germany) for the kind supply of PM2000. G.C., J.L.G.C., M.A.M., and S.P. would like to thank the European Commission (ALUSI Project, GROWTH-CT-1999/00083) for financial support. M.S.F., M.M., and G.R. would like to thank for Indas (Spain) for financial support.

References

1. B. R. D. GILLINGS, *J. Prosthet. Dent.* **45** (1981) 484.
2. B. R. D. GILLINGS, *ibid.* **49** (1983) 607.
3. O. OKUNO, in ''Third International Congress on Dental Materials'', edited by H. Nakajima and Y. Tani (Sheraton Waikiki, Hawaii, November 4–8, 1997) p. 53.
4. Y. TAKADA and O. OKUNO, *J. J. Mag. Dent.* **3** (1994) 14.
5. J. L. GONZÁLEZ-CARRASCO, M. C. GARCÍA-ALONSO, M. A. MONTEALEGRE, M. L. ESCUDERO and J. CHAO, *Oxid. Met.*, **55** (2001) 209.
6. Growth Programme ''Development of alumina forming ODS ferritic superalloys as new biomaterials for surgical implants (ALUSI)'', GRD1-1999-10659, Final Report.
7. J. CHAO, M. C. CRISTINA, J. L. GONZÁLEZ-CARRASCO and G. GONZÁLEZ-DONCEL, in ''Materials for Advanced Power Engineering, Proceedings of the 6th Liége Conference'', Vol. 5 Part II, edited by J. Lecomte-Beckers, F. Schubert and P. J. Ennis (Forschungszentrum Jülich GmbH, Central Library, 1998) p. 827.
8. J. L. GONZÁLEZ-CARRASCO, F. CEBOLLADA, M. C. CRISTINA and G. GONZÁLEZ-DONCEL, *J. Biomed. Mater. Res.* (1997) 453.
9. T. KULIK, H. T. SAVAGE and A. HERNANDO, *J. Appl. Phys.* **73** (1993) 6855.
10. M. AHMAD, M. C. CARTHY and G. GRONOWICZ, *Biomaterials* **20** (1999) 211.
11. B. D. CULLITY, ''Introduction to Magnetic Materials'' (Addison Wesly, Reading (MAL) 1972) p. 319.
12. I. WATANABE, K. HAI, Y. TANAKA, K. HISATSUNE and M. ATSUTA, *Dent. Mater.* **17** (2001) 217.
13. J. L. GONZÁLEZ-CARRASCO, M. L. ESCUDERO, M. COMÍN, M. F. STROOSNIJDER, G. CIAPETTI, G. BORCHARDT, S. GARCÍA and A. PILZ, in Proceedings (T39) of 17th European Conference on Biomaterials ESB2002 Barcelona, Spain, 11–14 de Septiembre, 2002.
14. G. CIAPETTI, L. SAVARINO, A. GIUNTI, S. PAGANI, J. L. GONZALEZ-CARRASCO, M.A. MONTEALEGRE, G. BORCHARDT and U. MILLER, in ''Proceedings (P44) of the

17th European Conference on Biomaterials ESB2002'', Barcelona, Spain, 11–14 de Septiembre, 2002.
15. G. CIAPETTI *et al*., unpublished results.
16. C. HENDRICH, U. NOTH, U. STAHL, F. MERKLEIN, C. P. RADER, N. SCHUTZE, R. THULL, R. S. TUAN and J. EULERT, *Clin. Orthop. Rel. Res.* **1** (2002) 278.
17. M. L. ESCUDERO and J. L. GONZÁLEZ-CARRASCO, *Biomaterials* **15** (1994) 1175.
18. M. C. GARCÍA-ALONSO, J. L. GONZÁLEZ-CARRASCO, P. PÉREZ P, V. A. C. HAANAPPEL, M. L. ESCUDERO, J. CHAO and M. F. STROOSNIJDER, *J. Mater. Sci: Mater. Med.* **12** (2001) 589.
19. R. W. LAAKMAN, B. KAUFMAN, J. S. HAN, A. D. NELSON, M. CLAMPITT, A. M. O'BLOCK, J. R. HAAGA and R. J. ALFIDI, *Radiology* **157** (1985) 711.
20. F. G. SHELLOCK and J. S. CURTIS, *ibid.* **180** (1991) 541.

Received 11 March
and accepted 3 December 2003