Carbon–carbon composite bearing materials in hip arthroplasty: analysis of wear and biological response to wear debris

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Ultra-high molecular weight polyethylene wear particles have been implicated as the major cause of osteolysis, implant loosening and late aseptic failure in total hip arthroplasties *in vivo*. This study initially screened 22 carbon–carbon composite materials as alternatives for UHMWPE in joint bearings. New bearing materials should satisfy certain criteria – they should have good wear properties that at least match UHMWPE, and produce wear particles with low levels of cytotoxic and osteolytic activity. Initial screening was based on wear resistance determined in short-term tribological pin-on-plate tests. Three materials (HMU–PP(s), HMU–RC–P(s), and SMS–RC–P(s)) which had superior wear resistance were selected for long-term testing. All materials had very low wear factors and SMS–RC–P(s), which had a wear factor of $0.08 \pm 0.56 \times 10^{-7}$ mm^3/Nm, was selected for the subsequent biological testing and particle size analysis. SMS–RC–P(s) showed good biocompatibility in bulk material form and also the wear particles had low cytotoxicity for L929 fibroblasts in culture compared to metal wear particles. Wear debris size analysis by transmission electron microscopy showed that the particles were very small, with the vast majority being under 100 nm in size, similar to metal wear particles. The potential osteolytic effect of SMS–RC–P(s) wear particles was investigated by culturing particles with human peripheral blood mononuclear cells and measuring TNFα production. SMS–RC–P(s) did not significantly stimulate TNFα production at a particle volume to cell number ratio of 80 : 1, indicating that the debris had a low osteolytic potential. The results of this study suggest that carbon–carbon composites, particularly those composed of PAN-based fibers may be important biomaterials in the development of next generation bearing surfaces for use in total joint replacements that have very low wear rates and reduced osteolytic and cytotoxic potential.

Introduction

Ultra high molecular weight polyethylene (UHMWPE) on metal or ceramic bearings have been used for the last 40 years in total hip arthroplasty and have provided good medium-term performance. However, in the long term, the problem of osteolysis and aseptic loosening has become a major concern in orthopedics [1, 2]. The mechanism by which this late failure occurs is dependent on wear of the UHMWPE and generation of micrometer and sub-micrometer wear debris [3–6]. During normal activity, such as walking or running, the metal or ceramic femoral head articulates on the polyethylene acetabular cup releasing UHMWPE wear particles into the periprosthetic tissue. These particles are not biodegradable *in vivo* and accumulate in the tissue where they are phagocytosed by tissue macrophages [7]. Particles in the 0.1–0.5 μm size range have been shown to activate macrophages to release numerous pro-inflammatory cytokines, of which TNFα has been suggested to be the main mediator of osteolysis [8–10]. These cytokines stimulate bone resorption through the activation of osteoclasts, resulting in osteolysis and loosening and the eventual failure of the implant [7, 11].

There is now considerable interest in alternative bearing surfaces that have significantly reduced osteolytic potential in order to overcome UHMWPE wear

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particle induced osteolysis. These new bearings should have the properties of reduced wear volume and generate particles with reduced biological activity. There has been renewed interest in metal-on-metal bearings, and these have been shown to have lower wear rates [12] and generate smaller particles in the order of 30 nm which have been found to have a reduced potential for osteolysis [13–17]. However, there have been other concerns associated with the particles generated by these bearings, such as the elevated release of metal ions which may cause cell death and tissue necrosis, increased risk of cancer of the lymphatic and hematopoeitic system, or development of hypersensitivity to the metal ions released [18–22]. Therefore interest in other novel materials to replace UHMWPE or metal as bearing materials for hip prostheses has increased.

We have previously shown that carbon–carbon composites may have the potential to satisfy the requirements of new materials for use in artificial joint bearings [23]. It was shown that carbon–carbon materials have lower wear rates than UHMWPE, and the wear debris produced in wear tests was very small with the majority of particles being less than 100 nm in size. These particles were not cytotoxic in *in vitro* cell viability assays. The aim of this study was to further investigate novel carbon–carbon composite materials with a view to identifying materials which had improved wear resistance, and produced wear debris that was small with low cytotoxicity. This study also investigated the response of primary human peripheral blood mononuclear phagocytes to wear particles produced from the novel carbon–carbon composite by measuring TNFα production to identify whether this material would have a reduced osteolytic potential *in vivo*.

Materials and methods

Materials

A series of 22 carbon–carbon composites with various combinations of fiber and matrix compositions were fabricated (Table I). Four different types of carbon fibers (SMS, HMU, P100 and P55) were incorporated into three different matrices (resin, resin-coke and pitch) in two types of carbon fiber reinforcement structures. The two types of reinforcement used were long fiber composites, which consisted of continuous carbon fibers aligned unidirectionally in the matrix, or short fiber composites, which consisted of short, chopped fibers arranged randomly within layers in the matrix. Of the four types of fiber, two were polyacrylonitrile-based (SMS, HMU) while the remaining two were pitch-based. Briefly, these materials were fabricated by bonding the fibers to the matrix to form disc-shaped green composites. These were carbonized at 1000 °C in a nitrogen environment, after which they were subjected to reimpregnation of the matrix under vacuum conditions to promote entrance of the matrix into the pores formed during carbonization. Reimpregnation was performed numerous times to ensure good impregnation of the matrix and to achieve good mechanical properties. During reimpregnation, a secondary matrix of pitch or resin could also be introduced. For example SMS–RC–P(S) is a composite composed of short SMS polyacrylonitrile-based fibers with a primary matrix of resin-coke and a secondary matrix of pitch. After fabrication carbon–carbon composites were machined into wear pins, with a 5 mm diameter bearing area.

Wear tests

Short-term wear tests were performed on all 22 composites using a multidirectional pin-on-plate tribometer. The wear pins were articulated against a smooth alumina ceramic counterface (mean initial Ra = 0.013 ± 0.003 μm) for one week, which was equivalent to approximately 330 000 cycles or 13.2 km. The sliding distance was ± 20 mm with a pin rotation of $\pm 40°$, cycle frequency of 1 Hz and an applied load of 180 N. The wear of the pins was measured gravimetrically using a microbalance (Mettler-Toledo, Switzerland), which was accurate to 1 μg.

Long-term wear tests were performed on three materials, HMU–PP, HMU–RC–P and SMS–RC–P. These materials were selected for long-term testing from the 22 initial materials because they had shown superior wear resistance in short-term studies. The wear tests were performed on a multidirectional pin-on-plate tribometer [24]. The wear pins were articulated against a smooth alumina ceramic counterface (mean initial Ra = 0.013 ± 0.003 μm). The test conditions were identical to those used in the short-term tests. Each test lasted three weeks or approximately one million cycles during which time, the counterfaces were visually inspected every week for signs of damage. No changes, including scratching, roughening or transfer film formation, were observed on the counterfaces during all the tests. Tests

TABLE I Carbon–carbon composite materials

Matrix	Type of fiber reinforcement	Fiber type				
		PAN-based		Pitch-based		
		SMS	HMU	P100	P100/P55 (50 : 50)	
–PP	Long	SMS–PP(L)	HMU–PP(L)	P100–PP(L)		
		Short	SMS–PP(S)	*HMU–PP(S)*	P100–PP(S)	
–PR	Long	SMS–PR(L)				
		Short	SMS–PR(S)			
–RR	Long	SMS–RR(L)		P100–RR(L)	P100/P55–RR(L)	
		Short	SMS–RR(S)		P100–RR(S)	P100/P55–RR(S)
–RC–P	Long	SMS–RC–P(L)	HMU–RC–P(L)	P100–RC–P(L)	P100/P55–RC–P(L)	
		Short	*SMS–RC–P(S)*	*HMU–RC–P(S)*	P100–RC–P(S)	P100/P55–RC–P(S)

P = pitch, R = resin, RC = resin-coke matrix, SMS = standard modulus surface treated, HMU = high modulus untreated, PAN = Polyacrylonitrile.

were lubricated with 25% (v/v) bovine serum in water with the addition of 0.1% (w/v) sodium azide to retard microbial growth. The lubricant was changed every week. After the tests were completed the wear pins were cleaned ultrasonically in detergent solution and isopropanol after which the pins were dried at 240 °C for 7 h. The pins were then stored in an environment with controlled temperature and humidity for seven days to stabilize their weight and wear was measured gravimetrically. The weight gain of additional soak control wear pins was determined to compensate for moisture uptake and all pins were presoaked for four weeks prior to the test.

Aseptic generation of carbon–carbon composite wear debris

Debris was generated on a pin-on-plate multidirectional tribometer similar to that used in the short- and long-term wear test except that the apparatus was housed in a class 1 laminar flow cabinet so that aseptic debris could be generated. The tribometer equipment was sterilized by heat at 180 °C for at least 4 h. Wear pins were pre-soaked in ultrapure water for irrigation (Baxter Healthcare, Newbury, UK) and sterilized by autoclaving prior to assembly in the tribometer, which was performed under stringent aseptic conditions. The lubricant used during the debris generation was 25% (v/v) fetal calf serum in Dulbecco's modified Eagles medium (DMEM) or Rosslyn Park Memorial Institute-1640 medium (RPMI) supplemented with 2 mM L-glutamine, 100 IU/mL penicillin and 0.1 mg/mL streptomycin (all Gibco, Paisley, Scotland). Debris was produced aseptically in cell culture medium so that the debris could be applied directly to the appropriate cells in culture. DMEM-based medium was used for culturing the particles with fibroblasts, whilst RPMI medium was used for peripheral blood mononuclear cell culture. The tribometer was run for 48 h which equated to approximately 13.5 km total sliding distance.

The majority of the debris generated was used in the cell culture studies except for a small aliquot which was retained for particle size analysis by TEM. Wear particles from this aliquot were isolated by centrifugation and treatment with potassium hydroxide. Briefly, the lubricant was diluted with an equal volume of sterile ultrapure water for irrigation and centrifuged at 2000 g in a benchtop centrifuge (MSE Mistral, Sanyo, Japan.) for 15 min. The supernatant was discarded and the wear debris was resuspended in 6 M KOH with sonication, which was then incubated for 48 h at 60 °C with frequent agitation. Wear particles were collected by centrifugation and washed twice with sterile ultrapure water. Finally, the particles were resuspended in ultrapure water and sonicated to ensure the particles had not aggregated. The wear particles were then prepared and characterized using transmission electron microscopy (TEM: Jeol 1200EX). Images of the wear particles were obtained and digitized for analysis and sizing using Image Pro Plus™ (Media Cybernetics, USA) and in-house software. Particle size and morphology were compared to those found for polyethylene pins tested under similar conditions.

Bulk material biocompatibility assay

Carbon–carbon composite SMS–RC–P bulk biocompatibility testing was performed to assess whether the material, in bulk form, was cytotoxic to L929 fibroblasts in culture. SMS–RC–P test discs were machined from the fabricated bars with the dimensions of 5 mm diameter and 5 mm cylinder height. The discs were cleaned in distilled water with sonication to remove debris from the machining and sterilized by autoclaving. The discs were placed into wells of a 12-well cell culture plate and attached in position with a 5 μL drop of collagen solution, which acted as an adhesive. Each test condition was performed in quadruplicate. Control wells were also included in the experiment these were; negative control 5 μL of collagen glue alone, while positive control was cyanoacrylate glue, which was known to be cytotoxic to cells in culture [25]. To each of the wells 150,000 cells were seeded in 1.5 mL of fibroblast culture medium (DMEM with 10% v/v fetal calf serum, 2 mM L-glutamine, 100 IU/mL penicillin and 0.1 mg/mL streptomycin). The cells were then cultured for 2 days at 37 °C, in 5% (v/v) CO_2 in air atmosphere after which the plates were examined under magnification. Cells were then either stained with coomassie blue to aid in the visualization for photomicrography or viable cells were stained using MTT. Briefly, for the coomassie blue stain, which stains all cellular proteins, cells were fixed in 10% (v/v) neutral buffered formalin for 20 min prior to staining with 0.1% (w/v) coomassie blue for 1 h and 2 × destaining with phosphate buffered saline (PBS). Finally, the cells were re-fixed with formalin and photographed. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma, Poole, UK) viability stain was performed by incubating the cells in 150 μL of MTT (5 mg/mL) for 3 h at 37 °C, washing in PBS and fixing in formalin.

L929 cell viability assays

Wear lubricant generated under aseptic conditions was dialyzed in 8000 Da cut-off dialysis membranes against fresh medium to remove any low molecular weight protein fragments which had been generated during the wear process. Low molecular weight protein fragments that may activate cells in culture have previously been shown to be generated by the mechanical agitation of wear lubricants independently of the load on the wear pin or volume of wear debris generated [26]. After dialysis the lubricant was diluted in serum-free cell culture medium to adjust the serum concentration to 10% (v/v) required for culturing cells. The volume of wear particles in the lubricant was calculated using the wear factors obtained from the long-term wear tests. For example,

$$\mathrm{WF} = \frac{\text{wear volume}(\mu m^3)}{\text{Load}(N) \times \text{sliding distance}(m)}$$

The medium was then diluted with fibroblast culture medium (containing serum) to obtain the required wear particle volume (μm^3) to cell number ratios (e.g. 80 : 1, 40 : 1, 4 : 1, 0.4 : 1, 0.04 : 1 and 0.004 : 1). L929 cells were seeded at 10 000 cells per well in a 96-well culture plate and cultured with the particles for five days at the

appropriate particle volume : cell number ratios at 37 °C in 5% (v/v) CO_2 in air. After 0, 1, 2, 3, 4, 5, days cell viability was measured using the ATP-Lite M assay (Packard, The Netherlands).

Cobalt-chrome metal particles at a ratio of 50 μm^3 per cell were included in the assays for comparison. These particles were also generated on a bidirectional pin-on-plate tribometer using distilled water as the lubricant. The mean size of the Co–Cr debris was 0.053 μm (range 0.024–2.97 μm) as determined by TEM and image analysis. Camptothecin (Sigma, Poole, UK) at 2 μg/mL was also included in each of the culture experiments as a positive control. This kills the cells by inducing apoptosis. Latex beads of 1 and 0.1 μm size (Sigma, Poole, UK) were also cultured with the cells as negative controls, to determine whether the process of phagocytosis of particles adversely affected cell viability.

Isolation of primary human peripheral blood mononuclear phagocytes

Peripheral blood mononuclear cells (PBMNC) were isolated using a method previously described [27]. Briefly, peripheral blood (20 ml) was collected from three healthy donors into preservative-free heparin and the mononuclear cell fraction isolated by density centrifugation over Lymphoprep (Nycomed Pharma AS, Oslo, Norway). The total number of viable PBMNCs were counted using trypan blue dye exclusion and the relative concentration of monocytes was determined using a latex bead ingestion assay [28]. PBMNCs were the seeded into U-shaped 96-well culture plates in PBMNC medium (RPMI-1640, 10% (v/v) fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin and 0.1 mg/ml streptomycin; Gibco, Paisley, Scotland) so that each well contained 1×10^4 monocytes. Cultures were then incubated overnight to allow for monocyte differentiation and attachement, after which the cells were co-cultured with wear particles.

Particle co-culture with PBMNCs

Wear lubricant was prepared as described for the L929 cell viability assays, except the medium used for PBMNC culture was RPMI-1640 based. The wear lubricant was dialyzed and the volume of wear particles generated calculated. The lubricant was diluted in serum-free medium to adjust the serum concentration to 10% (v/v) and further diluted in medium with 10% (v/v) FCS to obtain the various particle volume to monocyte number concentrations (80 : 1, 40 : 1, 4 : 1, 0.4 : 1, 0.04 : 1, 0.04 : 1 μm^3/cell) required. Each concentration was cultured with the cells in six replicate wells. Control wells were also included in each experiment. PBMNCs were cultured alone as negative controls. Positive controls were lipopolysaccharide (LPS, Sigma, Poole, UK) at 100 pg/ml and Palacos R wear particles at 100 : 1, which have previously been shown to stimulate cytokine production in PBMNC cultures [29].

PBMNC were cultured for 24 h with the particles at 37 °C, 5% (v/v) CO_2 in air prior to harvest of the culture supernatants for TNFα quantification by ELISA (Diaclone Research, Besançon, France). Cell viability was also determined by ATPLite assay to normalize the TNFα quantification for viable cell number.

Statistical analysis

Data were analyzed by ANOVA and the minimum significant difference between the individual means calculated using the *T*-method [30]. Data is represented by the means with 95% confidence limits.

Results

Wear tests

The short-term wear tests on the 22 carbon–carbon composite materials showed that three of the composites produced wear factors that were lower than $1 \times 10^{-7} mm^3/Nm$. These materials were HMU–PP(s), HMU–RC–P(s) and SMS–RC–P(s) and all were composed of polyacrylonitrile-based fibers.

The wear factors of the three materials from the long-term wear tests are shown in Table II. All three carbon–carbon composites showed significantly lower volumetric wear factors compared to polyethylene-on-alumina ceramic. HMU–RC–P(s) and SMS–RC–P(s) had lower mean wear factors than alumina-on-alumina, although they had large 95% confidence intervals associated with variability in water uptake which meant that the differences were not statistically significant.

SMS–RC–P(s), one of the lower wearing materials was selected for further study in bulk biocompatibility, wear particle size analysis and cell culture assays. It was chosen over the HMU–RC–P(s) because there was less variation in the wear results between replicate wear test pins.

Bulk material biocompatibility

SMS–RC–PS(s) was tested to assess whether the materials had any inherent cytotoxic activity to L929 mouse fibroblasts in culture. SMS–RC–P(s) test discs were not cytotoxic to the cells (Fig. 1). L929 cells had grown into a confluent layer around the SMS–RC–P(s) material and it was evident that cells were in close contact with the material. The cells had normal morphology, similar to the negative control (collagen glue only) wells and when the cultures were stained with MTT to show viable cells almost 100% of the cells were revealed to be viable. This was not the case in cultures treated with cyanoacrylate, which was the positive control. There was a marked absence of cells in proximity to the cyanoacry-

TABLE II Mean wear factors (± 95% confidence limits) of the carbon–carbon composites from long-term wear tests articulated against alumina ceramic counterfaces compared to UHMWPE-on-ceramic and ceramic-on-ceramic

Material	Mean wear factor ± 95% confidence limits $K \times 10^{-7} (mm^3/Nm)$
HMU–PP(s)	0.30 ± 1.13
HMU–RC–P(s)	0.069 ± 0.888
SMS–RC–P(s)	0.081 ± 0.562
UHMWPE-on-alumina	1.77 ± 0.77
alumina-on-alumina	0.20 ± 0.06

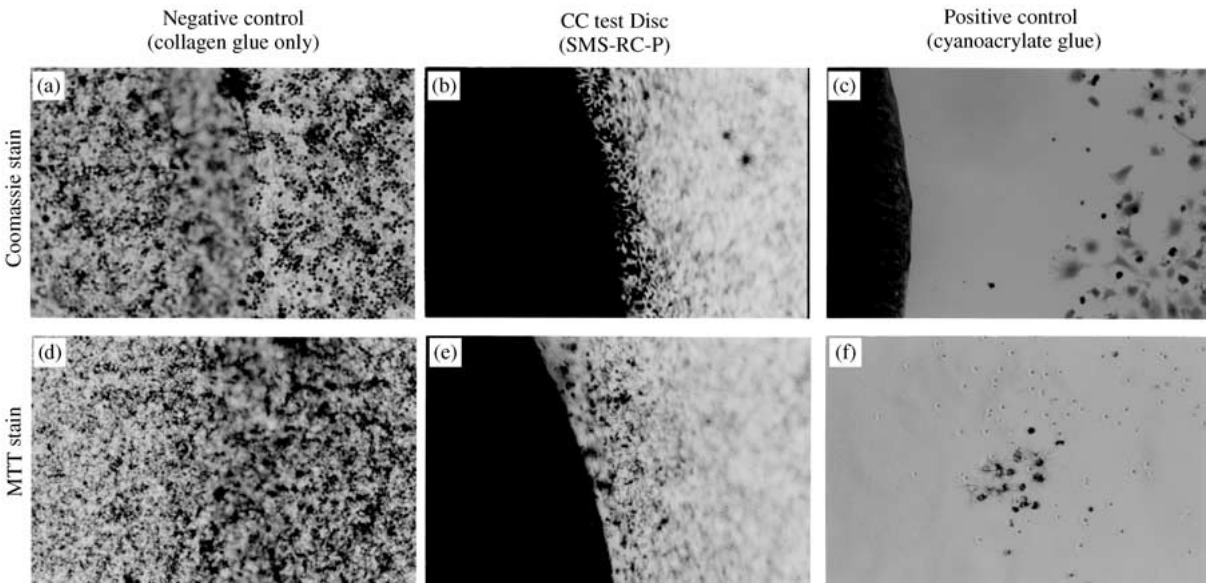

Figure 1 SMS–RC–P(s) Bulk material Biocompatibility test with L929 fibroblast cells; (a and d) collagen glue negative control; (b and e) SMS–RC–P(s) test disc; (c and f) cyanoacrylate positive control; (a–c) cells were stained with coomassie blue; (d–f) viable cells were stained with MTT (light photomicroscopy; magnification × 100).

late. Cells were only found at some distance from the cyanocrylate and these were sparsely spaced, had altered morphologies and after staining for viable cells using MTT the majority were found to be dead.

Particle size analysis

SMS–RC–P(s) particles were generated aseptically and a small aliquot was retained for wear particles size analysis. TEM analysis of the wear particles showed that SMS–RC–P(s) debris was small with the vast majority of particles being less than 100 nm in size (Fig. 2). The mode size of the particles was 47.8 ± 8.6 nm ($\pm$ 95% confidence intervals) and the size range was 22–440 nm.

L929 cell viability assays

In the cell viability culture experiments with L929 fibroblasts, SMS–RC–P(s) had no effect on cell viability at 4 μm^3 per cell and lower concentrations (Fig. 3). SMS–RC–P(s) at 40 μm^3/cell ratio showed a slight cytotoxic effect day 3 and day 4 where cell viability was near the 80% level compared to the untreated controls ($p < 0.01$, ANOVA). CoCr debris at 50 : 1 (50 μm^3 of debris per cell) and 5 : 1 significantly reduced cell viability ($p < 0.01$, ANOVA). CoCr at 50 : 1 induced a cytotoxic effect which was comparable to that of camptothecin (2 µg/ml), a chemical that inhibits cell proliferation and induces apoptosis. CoCr particles at 5 μm^3 per cell significantly reduced L929 fibroblast viability by approximately 60% relative to the control on day 3 and 4 and approximately 80% on day 5 ($p < 0.01$, ANOVA).

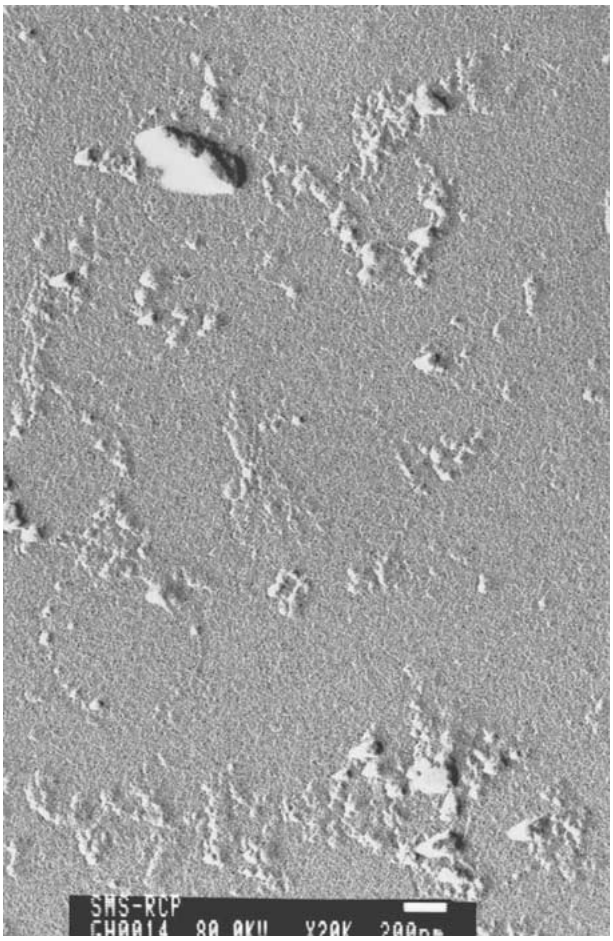

Figure 2 Transmission electron micrograph of SMS–RC–P(s) wear particles (magnification × 20 000, bar represents 200 nm).

Effect of wear particles on TNFα production in PBMNCs

SMS–RC–P(s) particles at various particle volume to cell number ratios were cultured with PBMNCs from three different donors for 24 h, after which the culture supernatant was collected and assayed for the pro-inflammatory cytokine TNFα. The production of TNFα by wear particle stimulated mononuclear phagocytes is shown in Fig. 4. Only the results from donor 3 are shown since all donors gave similar results. SMS–RC–P(s) at all the particle volume to cell number ratios tested did not stimulate TNFα production by mononuclear phagocytes

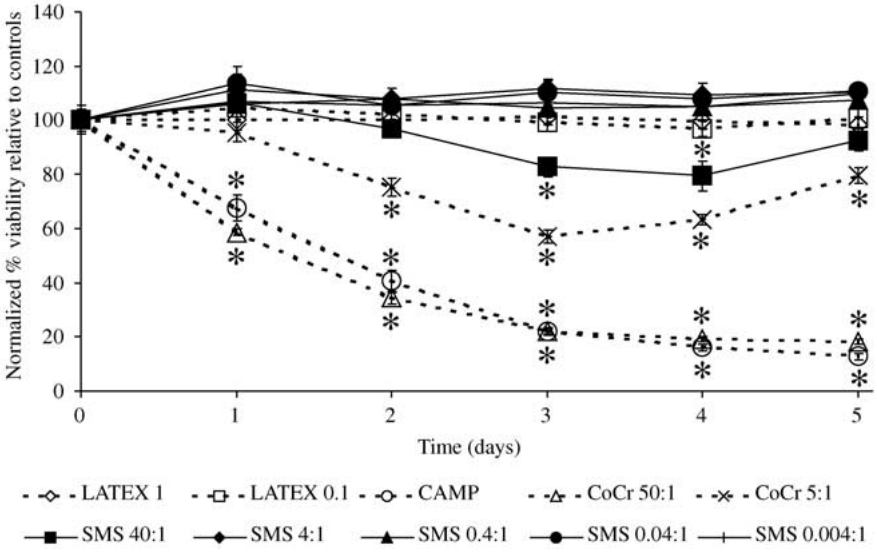

Figure 3 Percentage viability of L929 fibroblasts cultured with SMS–RC–P(s) particles at the indicated particles volume (μm^3) to cell number ratios compared to cells only controls ($n = 6$, $\pm$ 95% confidence limits, $^*p < 0.01$ ANOVA compared to cells only control).

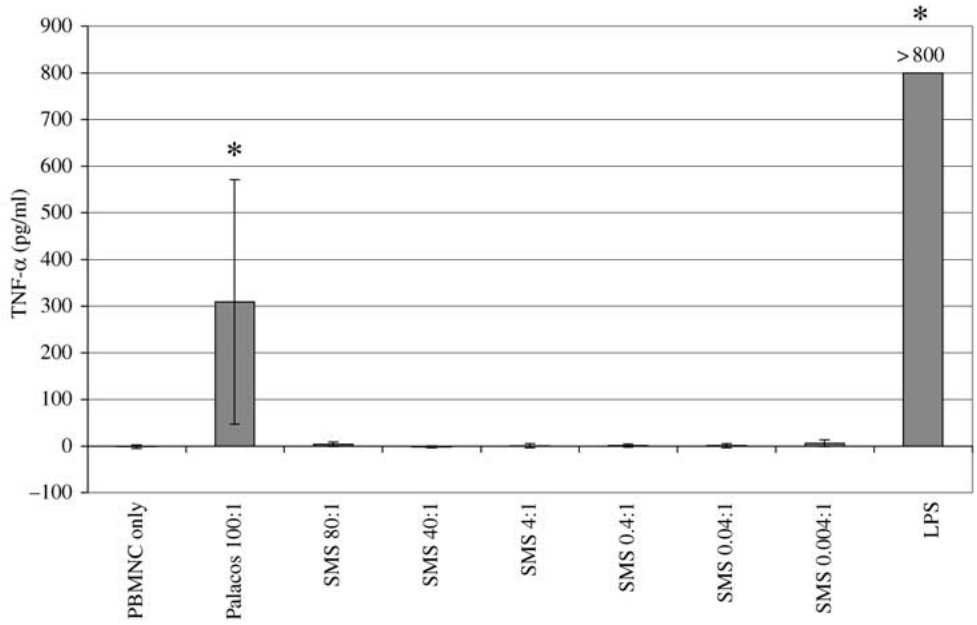

Figure 4 Production of TNFα by peripheral blood mononuclear phagocytes (donor 3) in response to SMS–RC–P(s) particles at the various particle volume to cell number ratios indicated ($n = 6$, $\pm$ 95% confidence limits, $^*p < 0.05$ ANOVA compared to PBMNC only control).

from any of the donors. Only the positive controls, Palacos R wear particles (100 μm^3 per cell) and LPS (100 pg/ml) significantly stimulated TNFα production ($p < 0.05$, ANOVA) compared to the cells only control.

Discussion

The generation of sub-micrometer and micrometer-sized UHMWPE wear debris and the subsequent biological reaction to the particles is currently believed to be the major cause of osteolysis and implant failure in total joint replacements. This has stimulated interest in developing new biomaterials for use in orthopedic prostheses, which overcome these problems.

We have previously reported preliminary studies to develop carbon-based composite bearing materials for use in artificial hip joints [23]. These composite materials had good wear properties and their wear particles were not in the biologically active size range and were shown not to be cytotoxic to cells in culture. The work presented here further investigated the potential of carbon-based composites for use in orthopedic implants and introduced novel carbon–carbon composites which had wear properties superior to those examined in the earlier study. The wear particles generated from these novel materials were also assessed for their effect on cell viability and also for their potential to stimulate an osteolytic response using an *in vitro* assay in which human peripheral blood mononuclear phagocytes were cultured with the debris.

Initially 22 carbon–carbon composites were tested for wear resistance in short-term wear tests. Of all these materials, three composites had superior wear properties

to UHMWPE and were selected for further study in long-term tests. These were composed of short fibers derived from polyacrylonitrile (SMS, HMU) surrounded by either pitch–pitch (P–P) or resincoke–pitch (–RC–P) matrices. The composites with –RC–P matrices had the lowest wear factors in the long-term tests, even when compared with ceramic-on-ceramic wear test results. SMS–RC–P(s) was selected for further study to investigate its bulk biocompatibility, cytotoxicity and osteolytic potential of wear particles. For the cell culture assays, the cells were treated with the particles at various particle volume to cell number ratios calculated from the wear factor results determined in the long-term wear tests. The highest particle volume (μm^3) to cell number ratio tested in the L929 cell viability assay was 40 : 1 and in the culture with PBMCs 80 : 1. Ideally a ratio comparable to the CoCr 50 : 1 control, in the L929 assay, and Palacos R 100 : 1 control, in the PBMC assay, should have been used. The lower wear rate of the carbon–carbon composites, however, limited the maximum concentration to 80 μm^3 per cell.

SMS–RC–P(s) was not cytotoxic to L929 fibroblasts in its bulk material or particulate form compared to the positive control materials. Particles generated in aseptic wear tests were very small, with the majority of particles being less than 100 nm in size. This compared well with previous work in which particles were generated from other carbon–carbon composite materials and the vast majority were found to be under 100 nm in size and not cytotoxic to L929 fibroblasts *in vitro*. The small size of the wear debris was similar to that of metal particles [13] but the cytotoxicity results indicated that carbon–carbon composite particles may not have the same potential for adverse tissue reactions and tissue necrosis *in vivo*.

The major biomaterials currently used in prostheses bearings are UHMWPE articulated against metal. This produces UHMWPE wear particles with the majority being 0.1–1 μm in size [5, 6]. These particles are believed to be responsible for inducing osteolysis and late aseptic loosening in hip arthroplasties. Clinically relevant UHMWPE particles stimulate PBMNCs to produce TNFα, a major osteolytic cytokine, *in vitro* [9, 10]. SMS–RC–P(s) particles were tested using a similar assay and were found to have no effect on TNFα production by PBMNCs. It was not possible to directly compare the carbon and UHMWPE particles in the culture studies due to their different densities: UHMWPE particles have a density lower than the culture medium and thus float, whereas the composite material particles do not. Thus different culture methods are used to test UHMWPE particles. Palacos R bone cement particles were therefore used as a positive control, since they have previously been shown to activate PBMNC and also have a density higher than the culture medium.

To summarize carbon–carbon composites such as SMS–RC–P(s) may be an important biomaterial in the development of future bearing surfaces for use in total joint replacements that have very low wear rates and reduced osteolytic and cytotoxic potential. SMS–RC–P(s) articulated against alumina had very low wear rates, much lower than UHMWPE-on-ceramic. Particles were very small, similar to metal wear particles but without the cytotoxicity associated with metal particles and did not elicit an pro-inflammatory response in culture with peripheral blood mononuclear cells.

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