Ultra High Molecular Weight Polyethylene/Graphene Oxide Nanocomposites: Wear characterisation and Biological Response to wear particles

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Abstract

In the field of total joint replacements, polymer nanocomposites are being investigated as alternatives to ultra high molecular weight polyethylene (UHMWPE) for acetabular cup bearings. The objective of the present study was to investigate the wear performance and biocompatibility of UHMWPE/graphene oxide (GO) nanocomposites. This study revealed that low concentrations of GO nanoparticles (0.5 wt%) do not significantly alter the wear performance of UHMWPE. In contrast, the addition of higher concentrations (2 wt%) led to a significant reduction in wear. In terms of biocompatibility, UHMWPE/GO wear particles did not show any adverse effects on L929 fibroblast and PBMNC viability at any of the concentrations tested over time. Moreover, the addition of GO to a UHMWPE matrix did not significantly affect the inflammatory response to wear particles. Further work is required to optimise the manufacturing processes to improve the mechanical properties of the nanocomposites and additional biocompatibility testing should be performed to understand the potential clinical application of these materials.

Keywords: Total joint replacements, UHMWPE, graphene oxide, wear, cytotoxicity, TNF-α
1. Introduction

Over the last decade, a number of carbon nanostructures, such as multi-walled carbon nanotubes (MWCNTs) or graphene nanosheets, have been investigated as potential fillers for polymer matrices due to their excellent tensile strength [1] and high thermal stability [2]. High-performance polymer nanocomposites with enhanced wear resistance and thermal stability, and superior tensile strength, have been developed by adding low concentrations (<2 vol %) [3] of carbon nanoparticles to polymer matrices [4-6] for use in medical applications. In the field of total joint replacements, polymer nanocomposites are being investigated as alternatives to ultra high molecular weight polyethylene (UHMWPE) for acetabular cup bearings. The biological response to wear particles, leading to osteolysis and eventual loosening of the implant [7], is one of the main issues associated with excessive amounts of UHMWPE wear debris generated during the lifetime of the artificial joint bearings. Recently, it has been acknowledged that these issues might be mitigated by using high-performance nanocomposites with enhanced tribological properties and higher oxidation stability.

Although there are currently alternative materials with several advantages over UHMWPE, these materials also have significant disadvantages. Metal-on-metal bearings have been shown to have almost 100-fold lower wear rates than metal-on-polyethylene bearings [8]. However, due to issues associated with the release of large numbers of metal particles and metal ion release, the use metal-on-metal implants has been associated with significant biological concerns and the implantation of this bearing combination has significantly decreased over the last five years [9]. Alternatively, ceramic bearings can be employed. Advanced ceramic materials, such as zirconia toughened alumina (ZTA), have been developed during the last decade, exhibiting extremely low clinical wear rates [10]. Ceramic-on-ceramic implants are the bearing combination of choice for younger and more active patients, but issues associated with implant instability, sudden brittle fractures or high cost, remain. Due to the concerns associated with metal-on-metal and ceramic-on-ceramic bearings, metal-on-polyethylene bearings are, to date, the bearing combination most commonly used; according to the UK National Joint Registry [11], more than 60% of hip bearings implanted in the UK in 2012 had a UHMWPE liner.

The wear performance of UHMWPEs has been improved by crosslinking of the polyethylene chains through gamma irradiation. However, the internal structure of UHMWPE is
compromised during the irradiation process, leading to a higher susceptibility to fatigue cracking and oxidation [12].

The addition of carbon nanostructures to UHMWPE matrices offers an alternative approach to improving the mechanical properties of bearing materials, which combines the numerous advantages of UHMWPE with oxidation stability over time. Previous investigations have suggested several advantages of the addition of MWCNTs and graphene oxide on UHMWPE [13]. For example, a study performed by Martinez-Morlanes et al. [14], demonstrated the antioxidant activity of MWCNTs, which would improve the oxidative resistance of UHMWPE. In addition, a study performed by Tai et al. [15] suggested that graphene oxide enhanced the mechanical properties of UHMWPE without affecting its biocompatibility. We have performed extensive mechanical characterization of UHMWPE/GO composites (0.1–2 %wt) and found that optimal mechanical properties were achieved with the addition of up to 0.5%wt GO, and above this level fracture strain and fracture toughness were significantly lower [16]. In addition, suboptimal dispersion of the GO within the UHMWPE matrix was observed above 0.5%wt GO [16]. To date, investigations concerning UHMWPE-based nanocomposites for use in hip replacements are very limited and, to the author’s knowledge, there are no studies which assess the tribological performance of these novel nanocomposites under hip kinematic conditions or assess the biocompatibility of the UHMWPE/GO wear particles generated due to articulation. The objective of the present study was to investigate the wear performance and biocompatibility of UHMWPE/GO nanocomposites.

2. Materials and methods
2.1 Materials
GUR 1020 UHMWPE/GO nanocomposites, with 0.5 wt% and 2 wt% GO, were investigated and compared with virgin GUR 1020 UHMWPE. Raw materials consisted of UHMWPE GUR 1020 powder (Ticona, Germany) and GO monolayer powder (Nanoinnova Technologies, Spain). UHMWPE GUR 1020 powder had an average molecular weight of 3.5x10^6 g/mol, density of 0.93 g/cm^3 and an average particle size of 140 µm. GO monolayer sheets had an average particle length of 3-5 µm and a thickness of 0.7-1.2 nm. An optimised ball milling technique [17] was used to prepare the UHMWPE/GO nanocomposites. Briefly, the following steps were followed to prepare the UHMWPE/GO mixtures; first, the required GO wt% content was dispersed in 30 ml of ethanol and blended with the UHMWPE powder. The slurry was then sealed in a zirconium oxide grinding jar containing zirconium oxide balls with a diameter
of 5mm. A planetary ball mill (Retsch PM 100, Germany) was used at a mixing speed of 400 rpm during 2 hours to prepare the UHMWPE/GO mixtures. These parameters have been previously identified as optimal conditions for preparing UHMWPE/GO nanocomposites [17]. After ball milling, ethanol was removed at 60 °C in an oil bath under stirring and the powder was kept at 60 °C in an oven during 24 h until it was fully dried. After sintering, cylinder-shaped wear pins with a contact face of 10 mm in diameter were machined from the UHMWPE/GO nanocomposites and the virgin UHMWPE bar stock.

2.2 Determination of wear factor of UHMWPE nanocomposites

Wear pins were soaked in distilled water for a minimum of two weeks to reach stable moisture content levels and stored for at least 48 hours in a temperature controlled environment. Before wear testing was carried out, wear pins were weighed using a digital precision balance (Mettler Toledo AT21, accuracy ±10 µg, UK). A well-established method using a six station multidirectional pin on plate wear simulator [18] was used to investigate the wear performance of the candidate nanocomposites and the reference material, non-irradiated GUR 1020 UHMWPE. High carbon (>0.2 % w/w) cobalt chromium alloy plates (ASTM F1537) with an average surface roughness of 0.01 µm were used during wear testing as counterface surfaces. Surface roughness was measured with a contact profilometer (Talysurf Series, Taylor Hobson, UK). The lubricant was 25% (v/v) bovine serum supplemented with 0.03% (w/v) sodium azide solution to minimise bacterial activity. Standard kinematic conditions, 160 N (nomical contact pressure= 2 MPa), ± 30° of rotation, frequency of 1Hz and a stroke length of 28 mm were used. Wear testing was performed for four weeks. Four pins of each material (UHMWPE/GO 0.5 wt%, UHMWPE/GO 2 wt% nanocomposites and non-irradiated UHMWPE GUR 1020) were tested during the process. Control pins of each material were used to control moisture content. Every week, the serum solution was renewed and the components of the rig were cleaned. The wear test lubricants were collected and stored at -20°C until required for particle isolation. At the end of each week, the weight loss of the pins was calculated and the wear factors were determined as follows:

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Wear\ factor\ (mm^3/Nm) = \frac{Volume\ loss}{Load \times Sliding\ distance}
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After collection, a sample of serum solution containing 1 mm³ of UHMWPE/GO nanocomposite particles was digested and particles were recovered and analysed according to the method described by Richards et al. [19]. Images of UHMWPE/GO nanocomposite wear
particles were taken using a high-resolution cold field emission scanning electron microscope (HITACHI SU8230).

2.3 Cell culture studies

2.3.1 Aseptic generation of wear particles

Wear particles from virgin UHMWPE GUR 1020, UHMWPE/GO composites to be used for cell culture studies were generated under aseptic conditions following a well-established protocol [20]. The efficacy of this method to generate sterile endotoxin-free wear particles for cell culture studies has been previously published by the authors [21]. In order to generate large volumes of wear particles, test pins were articulated against rough (Ra 0.07-0.08 µm) high carbon (>0.2 % w/w) cobalt chromium alloy plates. Wear pins were soaked, stored and weighed as previously described. Subsequently, wear pins, counterface surfaces and rig components were subjected to a thorough cleaning and sterilisation process. The cleaning and sterilisation protocol has been previously published [6]. A multidirectional pin on plate wear simulator operated under aseptic conditions and hip kinematic conditions was used to generate sterile wear particles. Each material was tested for one week. The lubricant used was RPMI 1640 medium (Lonza, UK) supplemented with 25% (v/v) bovine serum.

Possible microbial contamination was assessed by platting a small sample of lubricant into nutrient agar, heated blood and sabouraud agar plates, which were incubated at 37°C, 37°C, and 30°C, respectively, for three days. The sterility assessment was performed daily. After the tests, lubricants were stored at -20°C until they were required for cell culture studies. As described in the next section, cell cultures studies were carried out using L929 murine fibroblast cells and human peripheral blood mononuclear cells (PBMNCs). These cell populations have been shown to be appropriate models to be used in investigations of the biological response to UHMWPE, CoCr and ceramic wear particles [20-22].

2.3.2 Assessment of cytotoxicity of UHMWPE, UHMWPE/GO 0.5 wt% and UHMWPE/GO 2 wt% wear particles in L929 fibroblast cells

UHMWPE GUR 1020, UHMWPE/GO 0.5 wt% and UHMWPE/GO 2 wt% wear particles were cultured with L929 fibroblast murine cells. RPMI 1640 medium (Lonza, UK) supplemented with foetal bovine serum (10% v/v), L-glutamine (2mM), and penicillin-streptomycin
(100 µg/ml) (Invitrogen, UK), was used as culture medium for the cells. L929 cells were seeded at 1x10^4 cells/well into 96-well culture plates and cultured with wear particles at ratios of 0.005, 0.05, 0.5, 5, 50 and 100 µm^3 of debris per cell over five days at 37°C in 5% (v/v) CO_2 in air. Negative controls (cells only) and positive controls (camptothecin, a known inducer of apoptosis was used at a concentration of 2 µg/ml) were used as references. Six replicates were used for each test condition. Every 24h, the effect of the wear particles on the cells was assessed using the ATP-Lite assay (Perkin Elmer, UK) and the levels of ATP were determined in counts per second (CPS). The percentage cell viability was calculated taking the negative control as a reference.

2.3.3 Determination of tumor necrosis-α (TNF-α) release from human peripheral blood mononuclear cells (PBMNC’s) cultured with UHMWPE and UHMWPE/GO 0.5 wt% wear particles

UHMWPE/GO 0.5 wt% material was selected for cytokine release in PBMNCs as it had shown superior mechanical and wettability properties in previous investigations [16]. In addition, the authors have previously published a similar investigation of UHMWPE/MWCNTs (0.5 wt%), including tribological and biocompatibility performance [6], and in order to compare the two materials the same concentration of reinforcement material was selected for the present investigation. Ethical approval for the study was granted by the Faculty of Biological Sciences, Ethics Committee, University of Leeds (approval no. BIOSCI 10-018). Blood samples (approx. 30 ml) from three healthy donors aged 20-50 years were collected and stored the day before the experiment. In order to maintain the anonymity of donors and for traceability purposes, blood taken from donors was assigned a random number. At the time of this study, Donors 2, 3 and 15 were available and contributed to this work. On the day of the experiment, isolation of the mononuclear cells was performed by density centrifugation over Lymphoprep (Nycomed). The viability of the mononuclear cells was determined using the trypan blue exclusion assay.

Mononuclear cells were seeded at 1x10^5 cells/well into 48-well culture plates. RPMI 1640 medium (Lonza, UK) supplemented with foetal bovine serum (10% v/v), L-glutamine (2mM), and penicillin-streptomycin (100 µg/ml) (Invitrogen, UK), and RPMI 1640 medium (Lonza, UK) supplemented with 20mM HEPES and penicillin/streptomycin (100 µg/ml) were used as culture and transport medium, respectively, for the cells. An agarose gel technique [18] was employed to ensure that the wear particles could be cultured in the same plane as the cells.
UHMWPE GUR 1020 and UHMWPE/GO 0.5 wt% wear particles were resuspended in RPMI 1640 medium and mixed with a 2% (w/v) agarose solution (Gibco Life Technologies, Scotland) and cultured with the cells at a ratio of 300 \(\mu m^3\) of particles per cell. The positive control consisted of cells seeded with LPS at a concentration of 200 ng/ml. The negative control consisted of cells without particles. A second positive control was used and consisted of cells seeded with Fluorosphere\textsuperscript{TM} 0.2 \(\mu m\) particles (Invitrogen) at a ratio of 300 \(\mu m^3\) particles per cell. Four replicates were used per test. Seeded plates were incubated at 37°C in 5% (v/v) CO\textsubscript{2} in air for 24 hours.

Prior to determining the amount of TNF-\(\alpha\) released from the PBMNC’s, the viability of the cells was assessed using the ATP-Lite assay (Perkin Elmer, UK) as described previously. After this, TNF-\(\alpha\) production was measured using an ELISA assay (R+D Systems). Average optical density values were calculated at 450 nm and the TNF-\(\alpha\) concentrations of positive controls and samples in pg/ml were extrapolated from the lineal standard curve plotted using optical density values and standard concentrations.

2.4 Statistical analysis
One-way ANOVA and the Tukey method were used to determine significant differences in the wear rates of the materials investigated (UHMWPE/GO 0.5 wt%, UHMWPE/GO 2 wt% nanocomposites and virgin UHMWPE GUR 1020), the viability of L929 cells cultured with particles and its respective positive and negative controls, and the levels of TNF-\(\alpha\) between positive and negative controls and the PBMNC’s cultured with UHMWPE and UHMWPE/GO 0.5 wt% particles.

3. Results
3.1 Wear characterization
The wear factors (mm\(^3\)/Nm) of virgin UHMWPE GUR 1020, UHMWPE/GO 0.5 wt% and UHMWPE/GO 2wt% articulated against smooth (Ra=0.01 \(\mu m\)) high carbon cobalt chromium plates are shown in Fig. 1. The addition of 0.5 wt% GO nanoparticles did not have a significant effect on the wear behaviour of UHMWPE (ANOVA p>0.05). The addition of 2 wt% GO nanoparticles significantly enhanced the wear performance of UHMWPE, exhibiting a \(~30\%\) reduction in the wear rate (ANOVA p<0.05).
Examples of wear particles isolated from the UHMWPE/GO 0.5 wt% nanocomposite are shown in Fig. 2 (a-b). The wear particles isolated from the nanocomposite (GO 0.5 wt%) were similar in morphology to UHMWPE GUR 1020 wear particles reported previously [6].

3.2 Cytotoxicity of UHMWPE and UHMWPE/GO 0.5 wt% wear particles in L929 fibroblast cells

The effect of UHMWPE GUR 1020, UHMWPE/GO 0.5 wt% and UHMWPE/GO 2wt% wear particles on L929 fibroblast cells at ratios of 0.005, 0.05, 0.5, 5, 50 and 100 µm$^3$ of wear debris per cell over a five-day period is shown in Fig.3. The results presented in Fig.3 show the mean percentage viability compared to the negative control (cells only) at each time point. None of the materials showed any significantly adverse cytotoxic effects on the viability of the L929 fibroblast cells throughout the five-day period at any of the concentrations tested. The positive control (cells cultured with 2µg/ml camptothecin, a known inducer of apoptosis) used in the three experiments caused a significant reduction in L929 cell viability from day two onwards.

3.3 Determination of TNF-α release from human peripheral blood mononuclear cells (PBMNC’s) cultured with UHMWPE and UHMWPE/GO 0.5 wt% wear particles

The effect of UHMWPE GUR 1020 and UHMWPE/GO 0.5 wt% wear particles, 0.2 µm FluoSpheres and LPS on PBMNC viability from three different Donors (2, 3 and 15) at a ratio of 300 µm$^3$ of debris per cell over 24h is shown in Fig. 4 (A, C and E). PBMNC viability was not significantly affected by any particle treatment. The production of TNF-α by particle stimulated PBMNC’s from Donors 2, 3 and 15 is shown in Fig. 4 (B, D and F). PBMNC’s stimulated with FluoSpheres and LPS at a ratio of 300 µm$^3$/cell and a concentration of 200ng/ml, respectively, produced significantly higher levels of TNF-α over 24h compared to the cells only negative control, UHMWPE and UHMWPE/GO 0.5 wt% wear particle treatments. PBMNC’s stimulated with UHMWPE GUR 1020 and UHMWPE/GO 0.5 wt% wear particles released higher levels of TNF-α compared to the cells only negative controls, but the response was not statistically significant. No significant differences were observed between the level of TNF-α produced from PBMNC’s stimulated with UHMWPE wear particles and those stimulated with UHMWPE/GO 0.5 wt% wear particles for any of the donors investigated (Donor 2, 3 and 15).

4. Discussion
UHMWPE/GO nanocomposites have been identified as candidate bearing materials for total joint replacements. Previous investigations have shown that the addition of small amounts of GO nanoparticles improved the mechanical, thermal and wettability properties of virgin UHMWPE [21]. In the present work, the wear properties and biocompatibility of UHMWPE/GO nanocomposites have been investigated.

The addition of GO nanoparticles has been shown to have a positive effect on the wear performance of virgin UHMWPE [13, 15, 23]. However, the present study demonstrated that small amounts of GO nanoparticles (up to 0.5 wt%) did not improve the wear performance of UHMWPE; in order to achieve a ~30% reduction in the wear rate of UHMWPE, the addition of 2 wt% GO nanoparticles was necessary. This is in agreement with a study performed by Tai et al. [15], where a significant decrease in the wear rate of UHMWPE was only achieved after the addition of a minimum of 0.7 wt% GO, and approximately 35% reduction in the wear rate was achieved with the addition of 2 wt% GO. However, previous studies focussing on the mechanical characterisation of these UHMWPE/GO composites revealed adverse effects on some of the mechanical properties of the materials with greater than 0.5 wt% GO [16].

It has been hypothesised that only small amounts of nanoparticles can successfully be mixed to form homogeneous polymer nanocomposites, and that the addition of higher amounts can lead to embrittlement of the material [24], due to the formation of clusters in the structure of the material. These observations are in accordance with our previous findings, where addition of 2 wt% GO nanoparticles led to a decrease in the fracture toughness and fracture stress of the UHMWPE/GO nanocomposites, and disturbance of the fracture surface indicating particle agglomeration [16]. Therefore, although the ~30% reduction in the wear rate of UHMWPE due to the addition of 2 wt% GO nanoparticles is promising, the results should be interpreted carefully and further optimisation of the manufacturing process is required along with investigation into the effects of GO on other mechanical properties of the materials, such as fracture toughness.

Previous work has investigated the effect of different carbon nano-reinforcements on the wear performance of polyethylene [6, 25]. In the case of MWCNTs, 0.5 wt% MWCNT was necessary to achieve similar effects on wear resistance i.e. a ~30% UHMWPE wear reduction. These differences might be attributed to the ability of the nano-reinforcements to create bonding with the UHMWPE matrix. It has been demonstrated that different treatments such as surface modification and functionalization of the nano-fillers can lead to enhanced interactions.
between the fillers and the matrix [26, 27], resulting in an enhanced nanocomposite performance compared to that of materials prepared with unmodified nanoparticles. For example, Song et al. [2] studied the effect of GO nanosheets and γ-aminopropyl trimethoxysilane-modified GO nanosheets on the wear performance of poly (ether ether ketone) (PEEK). The results of this study demonstrated a further 15% reduction of the wear of PEEK/GO 0.1 wt% nanocomposites when GO nanosheets were surface treated. The authors attributed this effect to an enhancement of the GO/matrix adhesion by chain entanglement and chemical bonding between the nanosheets and the matrix. In order to achieve an optimum equilibrium between the amount of GO nanoparticles, mechanical properties and wear performance, further work concerning surface treatments of GO nanosheets should be considered.

In terms of cytotoxicity, UHMWPE/GO wear particles were not shown to have an adverse effect on L929 fibroblasts and PBMNC’s at any of the concentrations tested over both five-day and 24 hour periods, respectively. This is in agreement with our previous studies on UHMWPE/MWCNT composites [6]. However, other studies have reported negative effects of graphene on the viability of osteoblasts [28]. The results of this study performed by Lahiri et al. indicated a considerable reduction in osteoblast viability with increasing graphene concentrations [28]. To date, only a very small number of studies have focused on investigating the biocompatibility of GO and therefore more investigation is required in order to fully understand the effect of GO nanoparticles on human health.

In addition, the present study investigated the release of TNF-α from PBMNC’s stimulated with UHMWPE and 0.5 wt% UHMWPE/GO wear particles. PBMNC’s from three donors stimulated with 0.5 wt% UHMWPE/GO wear particles produced similar levels of TNF-α as those stimulated with virgin UHMWPE wear particles, indicating that the addition of 0.5 wt% GO does not cause an elevated inflammatory response in primary monocytes, although there was some variation across donors. Indeed, Matthews et al. [29] showed that PBMNCs isolated from different healthy volunteers could produce up to 15-fold differences in the level of TNF-α released after challenge with identical particles. Since the addition of high amounts of GO nanoparticles, i.e. 2 wt% GO, has been found to be detrimental to the mechanical properties of UHMWPE, the biological effects to 2wt% UHMWPE/GO wear particles were not investigated in this study.
The results of this study are promising in terms of GO biocompatibility, however additional work is required. The particles should be investigated at a range of volume doses, from low to high (10 – 500 µm³ per cell) to assess the effects of different levels of wear. Cytokine release should be measured at numerous time points, perhaps 8 and 12h, as well as at the 24h time point included in this study, to ensure that the cytokine response is not missed or underestimated. Our recent work has shown that although the response of PBMNCs to nanoscale UHMWPE particles at 12 and 24h was often similar in terms of incidence and magnitude, this was variable across donors and sometimes the peak cytokine response occurred at or before 12h [30]. The release of additional cytokines e.g. IL-1α, IL-6, IL-8 should also be investigated.

Although the concept of using UHMWPE carbon-based nanocomposites in orthopaedic applications is promising, and our previous studies support the use of these reinforcements in UHMWPE matrices, further work on the optimisation of the manufacturing process, optimal wt% of the reinforcement, and mechanical, tribiological and biological performance is required prior to these materials being translated to a clinical material.

5. Conclusions

This work has investigated the influence of graphene oxide nanoparticles on the wear performance of UHMWPE and assessed the biocompatibility of UHMWPE/GO wear particles. The main conclusions of this study can be drawn as follows:

1. The addition of GO nanoparticles to UHMWPE demonstrated a positive effect on the wear performance of UHMWPE.
2. The addition of small amounts of GO nanoparticles (up to 0.5 wt%) does not significantly alter the wear performance of UHMWPE; in order to achieve a ~30% reduction in the wear rate of UHMWPE, the addition of 2wt% GO nanoparticles was necessary.
3. UHMWPE/GO wear particles generated from 0.5 wt% and 2 wt% UHMWPE/GO nanocomposites have not shown any adverse effect on L929 fibroblasts at any of the concentrations tested over time. In addition, UHMWPE/GO wear particles generated from 0.5 wt% UHMWPE/GO nanocomposites have not shown any adverse effect on PBMNC viability at any of the concentrations tested over time.
4. The addition of 0.5 wt% GO to a UHMWPE matrix did not affect the inflammatory response to wear particles.
5. Further work into the optimisation of the manufacturing processes and biological responses to carbon-based nanocomposites are required to fully understand the potential clinical performance of these materials.

6. Acknowledgements

The authors would like to thank Kempe Stiftelserna (grant no. SMK-2932) and the Swedish Research School in Tribology for funding this project.

7. References


Captions:

Figure 1. Wear factors (mean ± 95% confidence limits) for UHMWPE GUR 1020, UHMWPE/GO 0.5 wt % and UHMWPE/GO 2 wt % against smooth high carbon cobalt chromium plates (*,** p <0.05 ANOVA)

Figure 2. UHMWPE/GO 0.5 wt% wear particles. Scanning electron micrographs of large flakes (magnification x20K and x9K)
**Figure 3.** Percentage viability of L929 fibroblasts cultured with (a) UHMWPE GUR 1020, (b) UHMWPE/GO 0.5 wt % and (c) UHMWPE/GO 2 wt % wear particles at the indicated particles volume (µm³) to cell number ratios compared to cells only controls. * Indicates significant reduction in cell viability compared to negative cell only control (p <0.05 ANOVA)

**Figure 4.** Viability of PBMNC’s and TNF-α release from PBMNC’s (mean ± std.dev.) from (a, b) Donor 2, (c, d) Donor 3 and (e, f) Donor 15, cultured with UHMWPE GUR 1020 and UHMWPE/GO 0.5 wt % wear particles, 0.2µm FluoSpheres at a ratio of 300 µm³/cell and LPS (200ng/ml)

* Indicates significant reduction in cell viability (a, c, e) and significantly higher TNF-α production (b, d, f) compared to negative cell only control (p <0.05 ANOVA)