Interactions between mammalian cells and nano- or micro-sized wear particles: physico-chemical views against biological approaches

by

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Abstract

Total joint arthroplasty (TJA) is more and more frequent approach for the treatment of end-stage osteoarthritis in young and active adults; it successfully relieves joint pain and improves function significantly enhancing the health-related quality of life. Aseptic loosening and other wear-related complications are some of the most recurrent reasons for revision of TJA. This review focuses on current understanding of the biological reactions to prosthetic wear debris comparing *in vivo* and *in vitro* results. Mechanisms of interactions of various types of cells with metal, polymeric and ceramic wear particles are summarised. Alternative views based on multidisciplinary approaches are proposed to consider physico-chemical, surface parameters of wear particles (such as: particle size, geometry and charge) and material (particle chemical composition and its nature) with biological effects (cellular responses).

Keywords: wear debris, biological reactions, nanotoxicity, morphology, TJR

Contents

1. Introduction

Lower limb implants represent an extensive orthopaedic market; the most recent annual figures for England and Wales showed number of total hip replacements (THR) to be 86,488 and 76,448 for total knee replacements (TKR) in 2013 [1] . With other aspects of lower limb surgery taken into consideration the estimated cost is £890 million each year for replacements in the UK alone [1] . Despite the economic burden, benefits to quality of life following this type of operation clearly indicate the need for such procedures [3] , [4] .

The leading cause for primary THR is osteoarthritis, accounting for between 60 and 83% of surgeries [5] , [1] . Although, this figure is dependent on the population characteristics, i.e. almost 90% of hip replacement cases attributed to osteoarthritis in the UK occur in a population over 50 years old [1] . However, other diseases also contribute to the need for revision but to a lesser extent; avascular necrosis (20%), fracture, traumatic arthritis (2.7%), congenital dysplasia (2.7%) and rheumatoid arthritis (5.5%) [5] In contrast, primary TKR is almost completely attributed to osteoarthritis (97%) thus little attributed to other diseases [1] . Osteoarthritis is a chronic, progressive disease affecting an estimated 50% of people over the age of 65 years with presenting symptoms of pain and poor function [7] ,[8] Large, weight bearing joints such as: the hip and knee are more commonly affected than other joints such as: shoulder, wrist and facet joints [9] . Constituents used in devices for joint replacements include a variety of materials such as: metals, polymers and ceramics or some

combinations of these. Currently, the most common articulation for THR and TKR are metal-on-polyethylene (Figure 1) [1] with the metal often being cobalt-chromium.

Implants can also be cemented or un-cemented depending on individual requirements. Bone cement is used to provide greater stability immediately after the operation and for an improved transition of stress from implant to bone [10] .

Despite widely reported successes, there are still failed procedures resulting in a combined total of 15,029 hip and knee implants being revised in England and Wales in 2011 [11] . The most commonly reported reason for failure is osteolysis, the loss of bone surrounding the implant that accounts for a staggering 50-70% of cases [12] , [13] . Hypersensitivity, aseptic loosening and instability are still included under the umbrella term of osteolysis, as they are all believed to contribute to loss of bone surrounding the implant [5] . Wear debris-induced osteolysis is a major cause of orthopaedic implant aseptic loosening, and various cell types, including macrophages, monocytes, osteoblasts, and osteoclasts, are involved.

Other reasons responsible for failures include: infections, dislocations, component fractures, subsidences, de-bonding of the cement mantle from the implant and environmental factors [14] ,[13] . Due to the most commonly reported reason for failure being wear debris induced osteolysis, there is a growing need to analyse the effects wear particles on cells found in the peri-prosthetic region.

2. Origin of wear particles

Wear debris is the result of friction between articulating implant components or between cement and implant, resulting in the release of small particulates. There are three types of wear mechanisms: fatigue, abrasive and adhesive [15] . The first is the caused by cyclic stresses inducing micro-fractures to occur within materials due to fatigue, once these micro-fractures reach the surface, wear particles are generated through delamination [16] ; delamination produces large ‘flakes’, often found from polyethylene knee implants [17] . Abrasive wear can be split into two sub-categories; two-body and three-body. Two-body

abrasive wear involves asperities of a hard surface in contact with a softer material; particulate is released from the softer material due to ploughing [18] . Three-body abrasive wear involves three materials instead; for example bone cement or fragments of bone between two articulating surfaces [19] . Finally, adhesive wear involves intermolecular bonds of the weaker material bonding to the stronger material, resulting in greater shearing. The Archard equation [20] ,[21] :

$$\Psi = k\frac{VW}{H} \quad (1)$$

Where:

Ψ is the wear rate,

k is constant

W is applied load

V is the sliding velocity

H is the hardness of the material

is generally employed as an indication of abrasive wear [20] , however, the micro-topography of the particle is neglected [22] . Finally, another form of wear generation *in vivo*, is corrosion; its products such as: chromium orthophosphate have been linked with failure of cobalt-chromium implants [23] [21] .

Classification of wear generation can also be assessed using a system developed by McKellop (1995) [24] , whereby there are four modes of wear particle generation (**Table 1**). The first mode is expected due to articulation between the two bearing surfaces, whereas subsequent methods of wear generation are not intended to occur.

3. Physico-chemical characterisation of wear particles (*in vivo* data)

3.1 Metal particles

Macroscopic findings during autopsy of asymptomatic and symptomatic patients revealed loss of vascular supply to the peri-prosthetic region [25] , likely resulting in necrosis of tissue. Peri-prosthetic tissue is often reported as being grey or black [26] , [27] suggesting a large quantity of metal debris present. However, the density of metallic staining of the tissue depends on the metal, with titanium alloy staining tissue darker than cobalt-chromium[26] , [28] . Microscopic findings reveal proliferation of macrophages in response to metal, ceramic and polyethylene debris [27] , [29] . The appearance of peri-prosthetic tissue has not only been found to be different depending on stability of the implant with stable implants without osteolysis have more aligned fibrous tissue compared to unstable implants with osteolysis [30] .

Metal wear debris accumulates in synovial fluid and tissues surrounding the implant [27] . Titanium-alloy and cobalt-chromium particles have both spherical and 'needle-shaped' wear debris [26] [31] [32] with the propensity toward spherical. Spherical titanium and cobalt-chromium wear debris are generally smaller than 14 µm [33] , [34] . However, determining wear debris < 0.2 µm largely depends on the resolution of the microscope used [31] . Needle-shaped wear or flakes seem to be much larger from titanium-alloy implants with the length being 10 - 400 µm, whereas cobalt-chromium debris was much smaller (6-767 nm) [34] , [26] , [32] . Doorn et al. (1998) [26] reported studies on isolation and TEM characterisation of wear particles from 13 tissues finding particles mostly rounded with sizes ranging from 51 to116 nm with little variation from patient to patient and/or a type of wear component or tissue. Another important consideration is the potential of agglomerates formation following isolation, providing a false impression of *in vivo* particles, as opposed to fixed retrieved tissue [26] .

Examining particles, through energy dispersive x-ray analysis (EDXA), from tissue collected at revision from 19 patients showed mainly chromium oxide and very few cobalt-chromium particles [32] , [28] . Chromium oxide particles were found to be slightly larger and oval in shape, whereas cobalt-chromium particles were small and dark [32] . About 10-15% of the debris were found to be needle shaped, whilst the remaining particles was closely split between rounded and elongated with a size of approximately 40 nm.

A growing concern over the biological effects of metal particles is aggravated by the evidences that immune system is taking up metal wear particles [36] . Of particular concern are the nanometer size metal particles that are capable of migrating to any part of body (excluding the brain) and depositing in lymph nodes, liver, spleen and bone marrow [36] [34] [21] . Recently, new data demonstrated the potential genotoxicity of metal wear particles [36] . Genotoxicity is the potential of a substance to cause harmful effects to the genetic material within a cell. If not corrected by the cell's normal repair processes, these effects can, in some circumstances, lead to cancer or birth defects. The Department of Health independent expert advisory committee, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) have identified that there is good evidence of damage to the genetic material in patients with hip implants that have components consisting partly or wholly of cobalt chromium or titanium alloys.

These studies indicate that chemical composition, size and shape of metal debris changed *in vivo*, this is important to consider when assessing information from *in vitro* studies.

3.2 Polymeric particles

Polymers employed in arthroplastry include polyethylene, often in the form of ultra-high molecular weight polyethylene (UHMWPE), poly-ether-ether-ketone (PEEK) and polymethylmethacrylate (PMMA). However, for this review, PMMA will be analysed as a separate entity due to PMMA being used as bone cement.

Wear debris from retrieved specimens showed ‘large irregular clefts’ in local foreign body giant cells ranging from less than 1 µm to 100 µm, believed to be due to the presence of polyethylene [38] , [39] , [27] . Wear debris retrieved from *in vivo* samples revealed polyethylene particles to be spherical with a diameter of 0.1–1.5 µm [40] , [41] , [35] . However, the polyethylene particles were also found in clusters connected with fibrils, 0.2-0.3 µm wide and up to 60 µm in length, with irregularly shaped edges [42] [43] . This is supported by characterisation of wear from tissue at THR revision where 43% were spherical granules <0.5 µm in diameter, whilst fibrils accounted for 35% and 10% were beaded particles [42] . The largest particles appeared to fulfil the criteria suggesting delamination of polyethylene [43] ; whereas the smallest particles were discovered within bone marrow [43] , potentially indicating hazardous effects on mesenchymal stem cells (MSC), which would then have a detrimental effect on the response to counteract osteoclast activity.

Not only is the shape of the particle varied upon retrieval, surface texture is also different. Analysis of UHMWPE particles using scanning electron microscopy (SEM) revealed 70% of particles to be globular, but not all particles were smooth as 36% of particles presenting a rough surface [44] . Similarly, 30% of retrieved particles were more needle-shaped, however, almost half had frayed edges and rough surface texture (determined using a basic arbitrary scoring system) [44] .

Wear debris from TKR and THR have slightly varying results with the proportion of particles being sub-micron greater in THRs [45] , [45] . Another aspect to consider in TKR is whether the implant is fixed or mobile, although, mobile knees make up a greater proportion than fixed knees (70% and 30%, respectively) [11] . Comparison between wear isolated from fixed-bearing knees revealed that they had a lower percentage of globular UHMWPE particulate debris than mobile-bearing knees [45] . Differences in particles found from implants could be an important consideration as to why there is lower aseptic loosening from TKR than THR implants [1] .

Despite PEEK showing promise as a new bearing surface for hip and knee replacements [47] , [48] , UHMWPE is still the polymer of choice. Surprisingly little information is reported on morphology of wear debris of PEEK implants found *in vivo*, even for spinal implants where PEEK is gaining application.

3.3 Bone cement particles

Bone cement particles range from being sparsely found to being detected in the majority of tissue from cemented implants [49] , [50] . However, this may be due to the isolation process dissolving PMMA and, therefore, the size of the particle can only be assessed by voids [49] . Sizes of voids have a large range (25-300 µm), which are surrounded by foreign body giant cells, with barium sulphate bone cement also detected in the voids [49] [50] .

Unfortunately, shape and surface of bone cement particles was not documented from in vivo studies However, in vitro a wear simulator study [51] where bone cement wear particles were generated showed sizes of around 670 micron.

3.4 Ceramic particles

Retrieved alumina wear showed the morphology of wear particles differed from metal due to lower number of fibrils and slightly higher number of granules and beads [42] . Wear debris analysed from revision revealed two distinct size groups depending on whether transmission electron microscopy (TEM) or SEM was used [52] . Wear analysed using SEM was found to be polygonal shaped with a mean approximately 0.4 µm and the largest particles being 3.2 µm [52] [50] , whereas the size obtained using TEM was 24 nm [52] . Interestingly, zirconia particles appear to be substantially smaller in size under SEM than alumina particles (0.28 µm and 0.44 µm respectively) [50] . Topography of particles also differs, as zirconia wear debris had smooth edges in comparison to alumina [50] .

3.5 Summary of *in vivo* studies

Metal and polymer wear debris have a wide size range in the nano and micron scale (Figure 2 and Figure 3). In contrast, ceramic particles have a very small proportion of micron-sized particles (Figure 2), but majority of particles are found in the nano scale (Figure 3). Finally, bone cement is entirely found in the micron scale (Figure 2 and Figure 3), which is much larger than ceramic and polymers, but within the range found of micron-sized metal particles. With regard to the shape of the material particles found in vivo, metals and polymers have both spherical and elongated particles. In contrast, ceramic particles were polygonal. Metals and polymers also share similar surface profiles with a mixture of rough and smooth particles as well as a mixture of particles with jagged or smooth edges. Ceramics show differing results within the material category, with zirconia revealing smooth edges whilst alumina revealed jagged edges.

Also wear debris morphology differs depending on implant material and its composition found *in vivo* studies Figure 4 and Figure 5.

4. Biological effects (*in vitro* studies)

4.1 Metal particles

In order to assess effects of metal particles on cells *in vitro*, a wide range of particle sizes have been used to emulate particles found *in vivo*. Large particles will likely represent flakes [35] , whereas smaller particles represent small, globular debris [33] [35] . In general, metal particles caused morphological changes, including disorganisation of the cell and reduction in focal contacts, dependent on size and number of particles ingested [53] [54] .

Cobalt has been shown to reduce cell viability at low concentrations (less than 25 µg/ml; furthermore it has been proven toxic to osteoblast cell lines and to inhibit their differentiation *in vitro* [53] .

Recently Dalal et al. (2012) [54] reported a statistically significant decrease in viability (> 90 %) for mouse osteoblast (MG-63) cell line when CoCrMo-alloy particle doses were increased to >50 particle/cell after 48 h.

Numerous studies showed the deleterious effects of exposure to Ti particles on mesenchymal stem cells (MSC) proliferation and differentiation [56] , [57] . However, the mechanistic aspects of these effects and their biological consequences are still lacking. In a recent study [58] , for the first time the Ti particle concentration range that reproducibly affected MSC functions without severely compromising cell viability, was established. The authors observed that exposure to doses >300 Ti particles/cell resulted in almost complete cell death, whilst at < 10 particles/cell, no detectable effects on MSCs were observed. The doses of 50 - 100 Ti particles/cell were a biologically useful range as the number of wear particles in macrophages of retrieved tissues from revision surgery is in the same range [31] Anatase TiO_2 nanoparticles have been described as potential candidate to improve the performance of some implants [59] [60] ; unfortunately they exhibited cytotoxicity on pre-osteoblasts and fibroblasts cells; these nanoparticles at concentration as low as 10 μg/ml were capable of inducing DNA fragmentation in MC-3T3 and L929 cells indicating on apoptosis that was later confirmed by flowcytometry. These results were also in a good agreement with previous studies performed with ZiO and TiO_2 nanoparticles.

4.1.1 Effects of micron-sized metal particles on macrophages

Direct comparison between cobalt-chromium and titanium alloy particles (1 - 3 μm) with rat peritoneal monocytes/macrophages revealed cobalt-chromium particles caused cells to become more rounded [61] , suggesting a decrease in focal contacts and potential cell death. In contrast, pure titanium particles had no effect on morphology [61] . Viability of human monocyte/macrophages (THP-1) cultured with titanium (1 – 3 μm) or chromium orthophosphate particles (1.42 μm) did not decrease [62] . However, cobalt-chromium particles (1 μm) and titanium alloy (1.3 μm) did decrease the viability of human THP-1

macrophages [54] . Metal particles (chromium orthophosphate, cobalt-chromium and titanium-alloy) caused a dose-dependent increase in proliferation of macrophages [54] , [62] .Therefore suggests certain chemical composites including corrosion products such as chromium orthophosphate, have differing effects. However, a greater number of macrophages would suggest a greater immune response. Effects of chromium oxide particles on macrophage proliferation is important to consider alongside systemic effects as serum levels are nine times greater than control [63] .

4.1.2 Effects of micron-sized metal particles on mesenchymal cells, osteoblasts and fibroblasts

Large micron-sized cobalt-chromium or titanium particles (10–15 µm) appear to have no effect on viability or morphology of cells obtained from a human osteosarcoma (MG-63 and SaOS-2) or mouse osteoblast-like cells [25] [53] . On the contrary, large micron-sized titanium alloy particles (8.9 µm) have been shown to induce a reduction in cell number of rat fibroblast-like cells [25] . However, cells proliferation was affected with a reported decrease with large metal particles (10-15 µm) [64] , suggesting that large particles may physically prevent proliferation. Large particles are relevant as particles of 400 µm have been found *in vivo* [35] . Nevertheless, despite the shape of the particles not being documented by Choi and co-workers (2005), images provided suggest the particles to be rounded and, therefore, may not be representative of flakes found *in vivo* as discussed in section 3.

Cytokines are also released from mesenchymal-derived cells when cultured with large micron-sized metal particles. Large titanium particles (21–85 µm) evoked a 5-fold increase in IL-8, but no increase in monocyte chemo-attractant protein (MCP)-1 from human osteoblast-like cells [64] . This suggests the increase in IL-8 would increase migration of neutrophils, but the lack of increase in MCP-1 would suggest migration of macrophages would not be influenced.

Culture of cells with smaller micron metal particles (1-5 μm) revealed damage to actin filaments in hMSC-like cells and MG-63 osteoblast-like cells [68] , suggesting a reduced ability migrate to the site of damage, likely inhibiting the repair process. However, [53] reported no change in morphology of MG-63 cells cultured with chromium (1.89 μm). Fibroblasts also showed morphology changes as bovine synovial fibroblasts cultured with cobalt became more rounded, whereas cells cultured on commercially pure titanium (cpTi) revealed an increased number of filopodia and ruffled membrane [5] . In contrast, titanium alloy or chromium did not induce a change in morphology to the same extent for bovine fibroblasts [5] . It has been reported that cobalt is more toxic than chromium [65] , despite this, analysis of retrieved tissue did not reveal any cobalt, but a greater number of chromium oxide particles and few cobalt-chromium particles [32] . Therefore, cobalt may be more toxic to cells, but does not appear to remain localised to the replacement.

Proliferation of both human and mouse osteoblasts, assessed through of ^{3}H-thymidine [55] [5] or through MTT assay, which provides an indication of metabolism of the cells. MTT assay indicated a decrease in proliferation of MG-63 osteoblast-like cells following incubation with cobalt particles (40% decrease), this was much greater compared to chromium particles (16% decrease)even with a much lower concentration of chromium [66] Interestingly, an increase in ^{3}H-thymidine was seen following culture of human fibroblast-like cells with titanium alloy (1.3 μm) particles in comparison to control, but cobalt-chromium alloy (1 μm) inhibited ^{3}H-thymidine uptake [55] [5] This suggests that cobalt inhibits proliferation, whereas other metal particles increase proliferation. Differences seen in the proliferative response could be attributed to cell type, chemical composition or shape. However, these titanium particles being globular and cobalt tubular [5] , do represent some particles found *in vivo* [31] .

Culture of human osteoblast-like cells obtained from patients undergoing primary surgery with titanium alloy, cobalt-chromium and cpTi particles showed slightly different results (mean sizes 2.8 - 4.4 μm) [68] . Titanium alloy particles did not produce a significant

reduction in viability (MTT), whereas cpTi produced a significant reduction [68] . However, viability of fully differentiated rat osteoblast cells decreased in a dose-dependent manner when cultured with titanium particles (3.1 μm) [68] . Cobalt-chromium particles had a greater reduction in viability compared to titanium [68] , again suggesting the greater toxicity of particles from cobalt-chromium implants. In contrast, viability assessed using lactate dehydrogenase (LDH) showed no significant decrease when chromium (1.89 μm) were cultured with MG-63 or SaOS-2 cells [53] . However, cobalt particles (4.75 μm) did produce a reduction in viability of MG-63 cells in a dose-dependent manner despite being much larger than chromium particles [53] . This may explain why particles of commercial cobalt-chromium produced a dose-dependent reduction in cells [55] , [69] as this will likely contain a mixture of chemical composites. An increase in apoptosis assessed by terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) of rat osteoblasts was found when cultured with cpTi particles (3.1 μm) [69] . This is an important finding because it indicates that cells undergo apoptosis as opposed to necrosis. In agreement, a murine osteocytic-like cell line, MLO-Y4, also showed an increase in apoptosis in culture with small micron-sized (1.2 μm) cobalt-chromium particles as shown by increased Caspase 3, 7 activity [70] .

Interestingly, interfacial wear simulated from rough stems caused a significantly greater reduction in viability of human osteoblasts when compared to wear from smooth cobalt-chromium stems [68] . This suggests that particles created from different implant surface profiles can have a bearing on results. However, the micro/nano surface profiles of the particles were not documented.

Cytokines such as IL-6, IL-8 and MCP-1 are increased following culture of cobalt with normal human osteoblast-like cells, however only IL-8 and MCP-1 are significantly increased [72] In agreement, increasing concentrations of titanium (< 5 μm) produced a non-linear response of IL-8 from normal human osteoblasts [73] . A much larger increase in IL-8 was seen with

smaller micron-sized particles suggesting smaller, endocytosed particles are likely to cause a greater reaction, but larger, non-ingested particles still evoke a response.

Cobalt and titanium alloy particles have no significant effect on the concentration of carboxy-terminal pro-peptide of type 1 pro-collagen (P1CP) when cultured with normal human osteoblast-like cells or those from patients undergoing total joint replacement [72] [68] . However, another study found contradictory results with type I collagen synthesis being reduced in a dose-dependent manner when metal particles were cultured with human osteoblasts and MSCs [72] [59] . Analysing the concentration of P1CP provides an indication of the turnover of type I collagen [75] , however this is not specific for bone. Once again, this is a concerning effect due to the fact that hMSCs cannot migrate, differentiate or produce collagen type I, all features needed for bone repair. However, a difference between MG-63 and SaOS-2 cells has been found with collagen type I synthesis being affected at a lower concentration of cobalt with the more mature SaOS-2 cells [72] , [69] . Despite the shape of particles not being assessed, the study does raise an important issue assessing the concentration of particles with cell types.

4.1.3 Effects of nano-sized metal particles

Morphological changes including enlarged vacuoles have been described when nano-sized cobalt particles (50 – 200 nm) were cultured with human dermal microvascular cells [76] . Human MSCs from patients undergoing primary revision showed a significant decrease in viability (using Trypan blue assay) when cultured with titanium particles (0.939 µm diameter) [56] [57] . Using titanium particles with a mean size 0.939 µm is relevant to the *in vivo* findings as particles are generally <2 µm provided they are not flakes [31] , [33] . In slight agreement with studies using micron-sized cobalt-chromium particles, spherical nano-sized particles induced a dose-dependent reduction in viability (MTT) of fibroblast-like cells, although, this was not so pronounced following assessment of LDH [70] . However, there are some important aspects to consider, aside from the assay. Firstly, both cobalt and chromium

were used and therefore it is not possible to differentiate between the chemical compositions. Also, the particles used were spherical whereas cobalt-chromium debris found *in vivo* in the nano scale was needle-shaped [26] , [34] . However, use of fibroblast cells might allow an insight as to the effects on fibrous tissue surrounding unstable implants.

Nano-sized cpTi particles (0.519 µm) cultured with hMSCs showed a decrease in proliferation and differentiation [76] , [59] . An increase in apoptosis of up to 12.7-fold in hMSCs, was found following culture with cpTi particles (0.519µm) [76] . Again, this would likely have an effect on the repair process if there are reduced numbers of MSCs in order to facilitate repair. Another important finding is that free radicals are released from fibroblasts upon culture with both micron and nano cobalt-chromium particles, however, nano particles caused a significantly greater DNA damage [70] . This adds to current speculation of a potential carcinogenic effect.

4.2 Polymeric particles

The majority of research of polymers *in vitro* uses polyethylene particles due to the vast numbers of implants using polyethylene, but also due to the theory that polyethylene particles are responsible for aseptic loosening [78] However, more recent studies are beginning to assess effects of PEEK particles [78] .

4.2.1 Effects of micron-sized particles on Haematopoietic cells

Small, micron-sized polyethylene particles (1.1 µm) cultured with the human monocytic cell line, THP-1 were not found within the cell and did not evoke a cytokine response [80] . In contrast, larger polyethylene particles (5.4 µm) cultured with J774 macrophages revealed a significant increase of TNFα and Prostaglandin E_2 [81] . These findings are interesting due to the fact that the smaller micron-sized particles did not evoke a response, but are within the range found *in vivo* [35] .

4.2.2 Effects of nano-sized particles on Haematopoietic cells

Large nano-sized polyethylene particles (0.1 - 0.7 μm) do not affect the viability of murine and human monocyte/macrophage cells, are able to induce a significant inflammatory response and differentiation to osteoclasts [82] , [82] [79] . The inflammatory response largely stems from the significant release of cytokines and chemokines (TNFα, IL-6 and prostaglandin E_2) following culture of large nano-sized particles (mean ranging between 0.24 - 0.45 μm) [83] . However, TNFα increased at a lower ratio of polyethylene particles:cell in comparison to Prostaglandin E_2 and IL-6 [83] . This suggests a potentially earlier release of TNFα from monocytes, which would increase osteoclastogenesis through the RANK/RANKL system. These are important findings as they add weight to the view that the sub-micron sized polyethylene particles are largely responsible for aseptic loosening.

4.2.3 Effects of micron-sized particles on mesenchymal cells

As with metal particles, polyethylene particles (1 μm) are found within the cytoplasm of MG-63 cells and human osteoblast-like cells [85] . However, polyethylene particles were also found attached to the cell membrane and morphological changes of MG-63 cells included an increased number of cell extensions and a ruffled cell membrane [85] . In contrast to results found with titanium, ^{3}H-Thymidine incorporation was increased following culture of MG-63 cells with UHMWPE (1 – 6 μm) [86] . This suggests that polyethylene particles actually increase proliferation of osteoblast-like cells. Polyethylene particles (2-3 μm) cultured with osteoblasts also increased the level of MCP-1, suggesting an increased signal for migration of macrophages [45] . Interestingly, different topography of particles evokes different responses. Elongated particles (9.9-26.9 μm) appear to have a greater IL-1β and TNFα production than smaller, globular particles (5.6-9.55 μm) from mouse synovium-like cells [78] . Elongated particles also induce a significantly greater number of apoptotic cells and thickening of the mouse synovium-like membrane in comparison to both control and globular particles (0.972-1.94 μm) [44] . Cytokines such IL-1β and TNF-α are significantly increased

with rough-globular, rough-fibrillated and smooth-fibrillated particles [44] , thus suggesting that the surface profile of the particle is an important aspect.

Due to the relatively new use of PEEK for orthopaedic implants, little is reported about the effect on mesenchymal-derived cells *in vitro*. However, no change in cell morphology or viability was noted with mouse-3T3 fibroblast-like cells cultured on PEEK discs [87] , [88] . Also, nanoparticles (27.7-90.2 nm) obtained from different compositions of PEEK were found to be less cytotoxic to L929 cells as compared to cobalt-chromium wear particles [89] .

4.3 Bone cement particles

Both cemented and uncemented implants are inserted each year. Interestingly, knee implants have a much greater percentage of cemented implants (88%) than uncemented [1] . However, hip implants have a greater percentage of uncemented implants, unless the sample population is greater than 70 years [1] . As bone cement particles are found *in vivo* [49] [50] , it is important to assess the effects of these particles on cells found in the peri-prosthetic region.

4.3.1 Effects of bone cement particles Haematopoietic cells

Both PMMA and calcium phosphate bone cement had no effect on the viability of U937 monocytic cell line [90] . However, retrieved debris consisted of irregular, globular particles and sparse agglomerates [90] , which may not have a detrimental effect to bone formation following cytokine release compared with particles with a rough surface and elongated shape, as found with polyethylene particles [44] . Significantly, retrieved debris from wear testing was collected on 0.1µm membranes and therefore would not include nanoparticles less than 100nm, which may have had a different effect.

4.3.2 Effects of bone cement particles on mesenchymal cells

Commercially obtained PMMA cultured with rat osteoblast-like cells, MG-63 and SaOS-2 cells were less apoptosis-inducing than titanium particles [69] . Filtration of PMMA through a

25 µm membrane and culture with fully differentiated human osteoblasts significantly reduced uptake of ^{3}H-thymidine to nearly half of the particle-free control samples [91] . The results also reveal an increase in the osteocalcin and IL-6 release from osteoblasts [91] . Therefore, it is important to consider whether implants are cemented when analysing results from particles obtained *in vivo*.

Calcium phosphate bone cement also causes a dose-dependent reduction in viability and proliferation in rat osteoblast and fibroblasts [65] . However, the smaller micron-sized particles (range 0.79-14.25 µm) had a greater detrimental effect than the larger micron sized (range 3.81-65.44 µm) particles [65] . The large particle range of bone cement particles will likely represent *in vivo* sized particles represented by voids found upon isolation [49] , [50] . However, the smaller range, with a greater detrimental effect is not in the range found *in vivo*.

4.4 Ceramic particles

A growing number of ceramic implants are being used due to the low wear properties of zirconia and alumina [92] . However, the majority of particles are found within the nano-size range [52] , therefore information on the effect of these particles have on cells is important to determine in comparison with micron-sized particles from other materials.

4.4.1 Effects of nano-sized particles on Haematopoietic cells

Large nano-sized zirconia or alumina particles (0.3-0.5 µm) had no effect on viability of THP-1 macrophages or human U937 monocytic cell line, but did induce a decrease in proliferation [55] . The THP-1 cells also showed an increase of IL-8 and IL-1β [55] . Interestingly, alumina particles do not promote the differentiation of macrophages to functional osteoclasts, but actually reduce differentiation by altering the c-fos expression [82] . This suggests that alumina particles may not contribute to aseptic loosening in comparison to other materials.

4.4.2 Effects of micron-sized particles on mesenchymal cells

Using osteoblast-like obtained during revision THR, exposure of large alumina (<80 µm) particles decreased cell growth [93] , similar to large micron-sized metal particles [64] . However, the reduction was not as substantial as with polyethylene particles (<80 µm) [93] . Although, very large ceramic particles were not found *in vivo* [52] , therefore, the ceramic results are not necessarily attributable to a clinical context.

In contrast to the large 12.7 fold increase following culture of hMSCs with titanium, zirconia particles evoked a smaller 5.5 fold increase in apoptosis [57] . This suggests that at the same concentrations, titanium particles have a greater detrimental effect on hMSCs than zirconia particles.

4.4.3 Effects of nano-sized particles on mesenchymal cells

Exposure of Alumina particles with L929 mouse lung fibroblasts with bimodal ranges 0.3-0.4 µm and 5-20nm had no effect on viability [94] . However, significant dose-dependent reduction in viability of hMSCs was found when cultured with zirconia [57] . In contrast, no reduction in viability (MTT) was seen when zirconia particles (0.5 µm) were cultured with osteoblasts from bone marrow of patients undergoing THR or MG-63 cells [55] [68] . Interestingly, exposure of Human osteoblast and chondrocytes for a short time period (2 and 6 hours), revealed nanoparticles had a greater detrimental effect on cells when compared to the larger particles [94] . This was also found following culture with Alumina particles (mean 23nm and agglomerates of 650 nm) [94] . The short exposure time may indicate that nanoparticles have an earlier effect.

4.5 Summary of *in vitro* findings

4.5.1 Haematopoietic cells

Viability of haematopoietic cells depends on size and chemical composition of particles. Micron-sized titanium alloy and cobalt-chromium decreased viability, whereas micron-sized cpTi, chromium orthophosphate or bone cement did not decrease viability. Micron-sized cpTi particles were shown to induce a release of Prostaglandin E_2 and IL-6 from human peripheral blood mononuclear cells, but no increase of IL-6 was found following culture of THP-1 cells with cobalt-chromium or titanium alloy. With polymers, contradictory results are found as to whether TNFα and Prostaglandin E2 are released following culture of small micron-sized particles.

In contrast to the micron-sized particles, nano-sized polymeric or ceramic particles do not decrease viability of haematopoietic cells. Proliferation of haematopoietic cells are increased following culture with nano-sized ceramic particles but also increased with micron-sized metal particles. Nano-sized polymer particles were shown to induce an increase in TNFα, IL-6 and Prostaglandin E_2, whereas nano-sized ceramic particles were only shown to induce an increase in IL-8.

4.5.2 Mesenchymal cells

Small micron-sized metal and polymeric particles cause morphological changes to cells. However, this was not found with large micron-sized particles. Large micron-sized metal, ceramic and bone cement particles all decreased proliferation. Contradictory results were found with small micron-sized particles with polymers, titanium and cobalt decreasing proliferation of osteoblast-like cells, but titanium increased proliferation of rat fibroblast-like cells. Viability of cells tended to have a greater decrease following culture of micron-sized particles of cobalt in comparison to other materials. However, viability also decreased following culture with large micron-sized bone cement. Large metal particles induce IL-8 release, whereas large bone cement particles induce IL-6 release from mesenchymal-

derived cells. Smaller micron-sized cobalt particles induced IL-6, IL-8 and MCP-1 release, whereas small micron-sized titanium particles only induced IL-8 release. Small micron-sized particles also increase the concentration of MCP-1 from mesenchymal-derived cells.

Nano-sized metal particles also decrease viability of mesenchymal-derived cells. In contrast to micron-sized particles, nano sized particles increase free radicals and chromosomal damage. Nano-sized ceramic particles have contradictory results with zirconia reported to cause a dose-dependent decrease viability of hMSCs, but no effect on L929 mouse lung fibroblasts, human osteoblasts or MG-63 cells. However, another indication found using ceramic studies is the potentially earlier effect of nano-sized particles.

Cell proliferation and differentiation are affected by the size, shape, and chemical composition of the wear particles as shown in Figure 6.

5. Conclusions

The wear particles isolated from wear simulators and periprosthetic tissues appeared to be different in size and shape [96] despite some authors justify that *in vitro* tribological studies are relatively closely reproduce *in vivo* results. Another issue is that wear debris size and shape is particle characterisation tool dependent. Therefore, a wide distribution of sizes and shapes for the same implant material is found in literature. In addition, wear debris particles morphology is influenced by limitation of experimental precision of quantitative techniques.

Also. wear debris is distributed not homogeneously throughout the tissues due to clumping and clearing of the debris through drainage. Therefore, the number of wear particles collected per unit of wet tissue is highly dependent on the biological variations of the tissue [97] .

The wear debris size are ranging from nanometers to micrometers, varied in shape and volume depending on the type of joint (knee/hip or mobile/fixed), material type, implant

design, wear mechanism, and experiment conditions (load, speed, and lubrication). The most common debris shapes found are globular, fibrilar, flake and needle.

As general trend, wear particles retrieved irrelevant on material (polyethylene, metal and ceramics implants ext) showed higher inflammatory response to living cells when they were smaller in size. In addition, phagocytosis of particles is found to be debris-sized-dependent. Therefore, the nano-sized wear particles retrieved from any prosthesis material are expected to be highly capable of stimulating cells at a given high volumetric dose. The size-dependent response rate weakens with lower doses [98] .

To date only a few studies consider wear particles composition and surface charge effects on interaction with cells. However, nanotexture of wear particles, their size, shape and surface charge as well as chemical composition should be studied in complex to determine how all these properties effect biological activity of cells. Such *in vitro* studies would be a more realistic insight on processes occurred *in vivo*. In conclusion, a schematic route from surface and material properties of the implant device to implant failure is given in Figure 7.

This review highlights the need to have a complex approach by considering physico-chemical, surface parameters of wear particles (such as: particle size, geometry and charge, particle surface area distribution, surface patterning, morphology, surface composition, particle surface-to-cell surface area ratio, particle mass dosage, direct toxicity through cellular phagocytosis ion exposure and hydrophobicity/hydrophilicity), material (particle chemical composition and its nature) and mechanical (interaction forces) properties with biological effects (cellular responses).

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7. References

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Table 1. System developed by McKellop (1995) in order to further categorise wear debris.

Mode 1	Wear debris as a result of the articulation between the two bearing surfaces intended to be in contact within the joint
Mode 2	Wear debris formed as a result of the two intended bearing surfaces articulating unconventionally
Mode 3	Third body wear causing release of wear debris
Mode 4	Backside wear as a result of materials within a component articulating, thus causing release of wear debris

8. Figures caption

Figure 1 Proportions of each articulation used for hip implants.

Figure 2 Median size for different shapes of wear particles of various implant materials retrieved from *in vivo* (a); zoomed (nanoscale) for metal wear particles (b).

Figure 3 Typical morphology of debris from periprostetic tissue (a) UHMWPE, (b) alumina, (c) spherical (UHMWPE), (d) sheet/flake (UHMWPE) and (e) fibril (UHMWPE).

Figure 4 SEM images of PEEK-Optima, UHMWPE and X-UHMWPE.

Figure 5 SEM image of CoCr wear particles.

Figure 6 Size dependent biological response of wear particles taken from [98] .

Figure 7 Schematic representation of the route from surface and material properties of the device to implant failure.

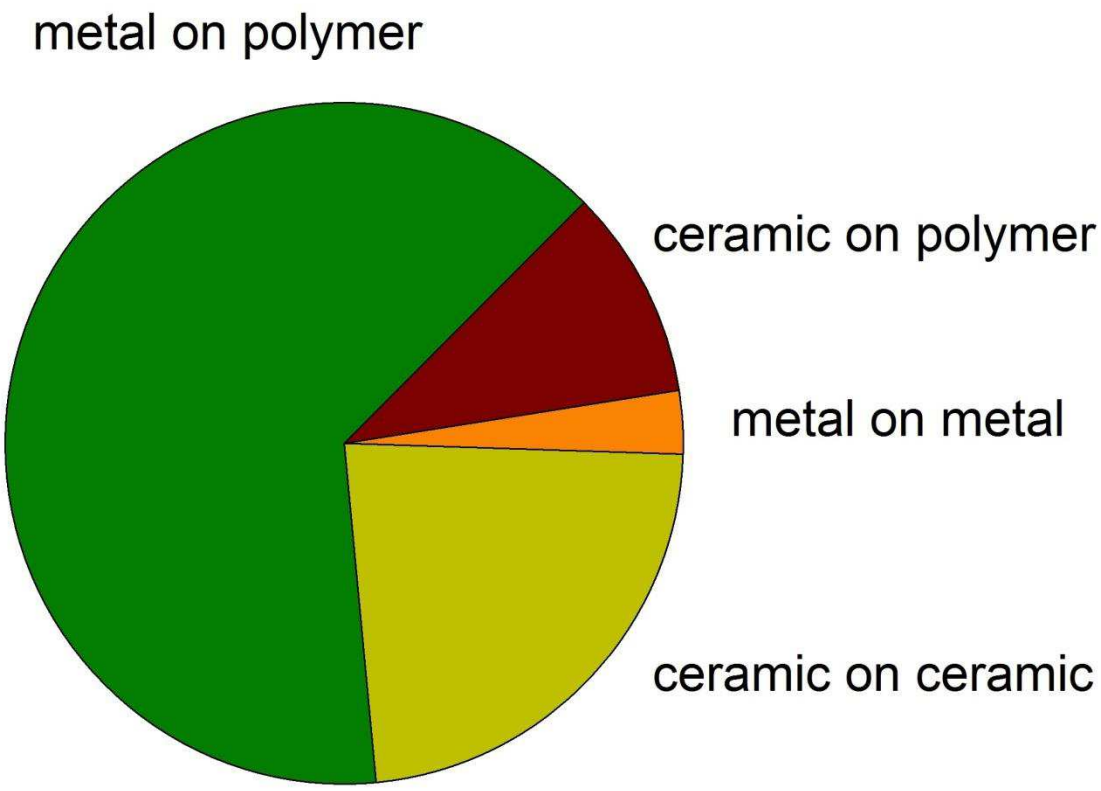
metal on polymer
ceramic on polymer
metal on metal
ceramic on ceramic

Figure 1

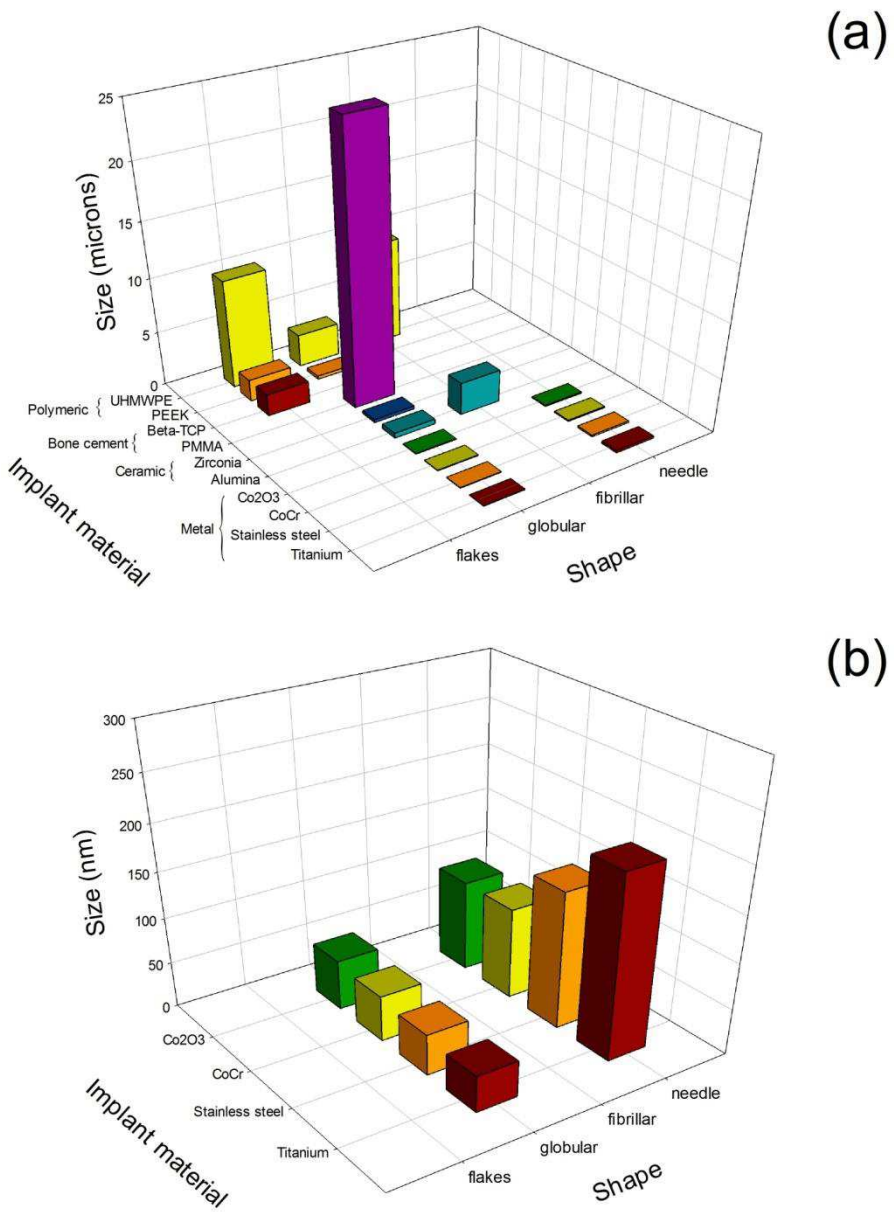
(a)
Size (microns)
25
20
15
10
5
0
Polymeric
UHMWPE
PEEK
Bone cement
Beta-TCP
PMMA
Ceramic
Zirconia
Alumina
Metal
Co2O3
CoCr
Stainless steel
Titanium
Implant material
flakes
globular
fibrillar
needle
Shape
(b)
Size (nm)
300
250
200
150
100
50
0
Co2O3
CoCr
Stainless steel
Titanium
Implant material
flakes
globular
fibrillar
needle
Shape

Figure 2

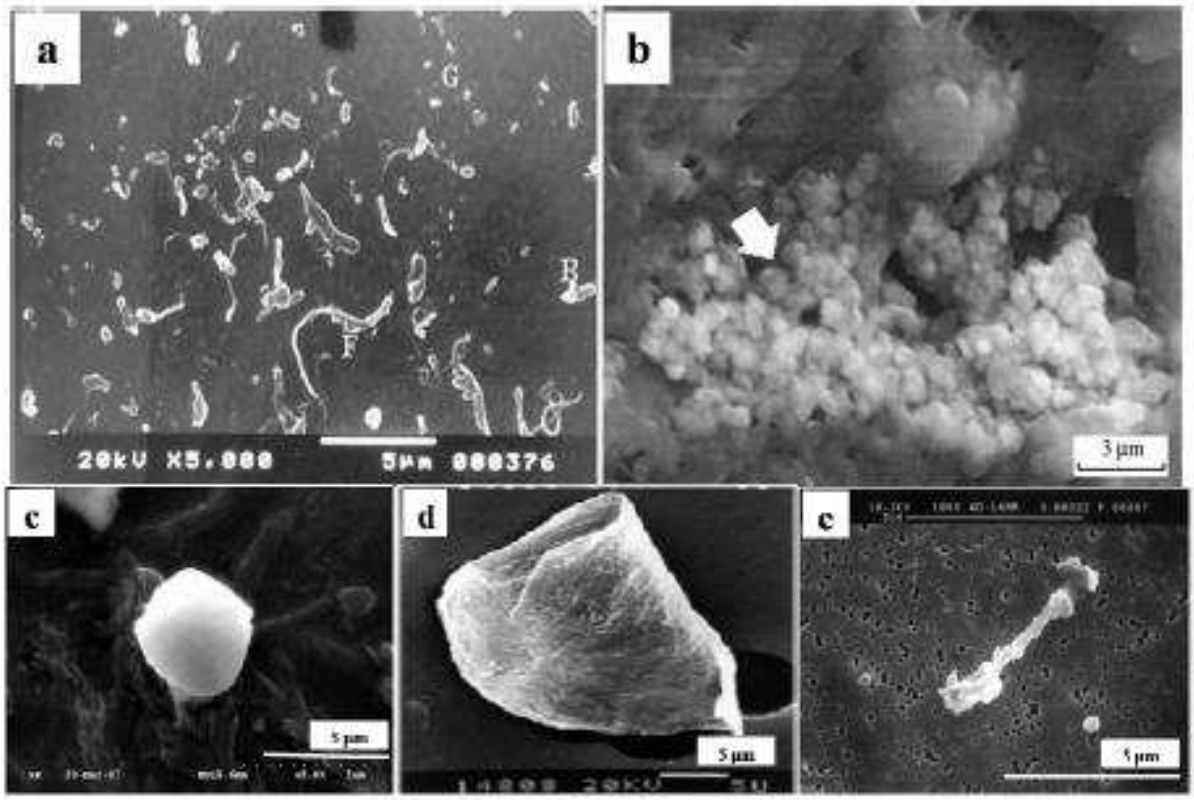

Figure 3

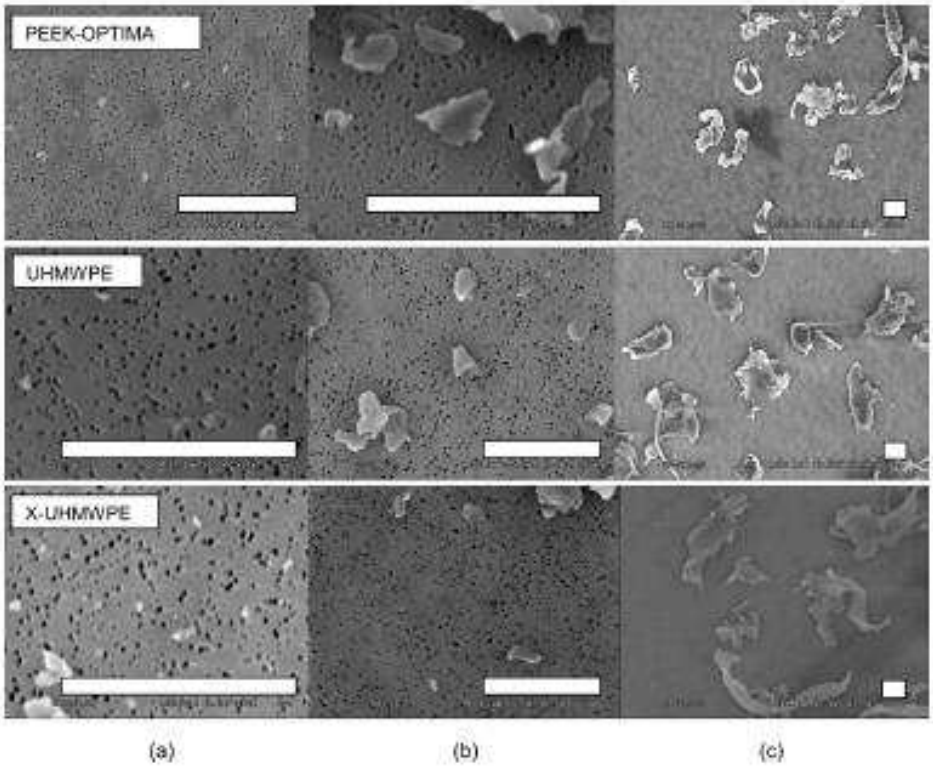

Figure 4

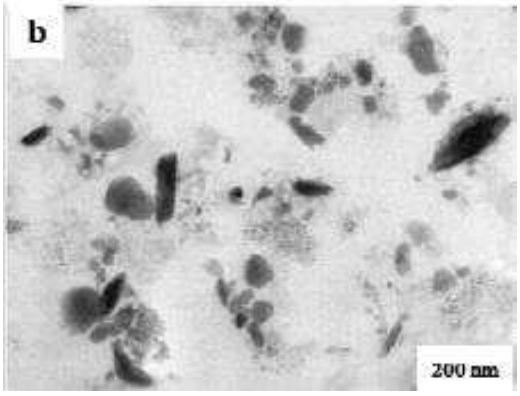

Figure 5

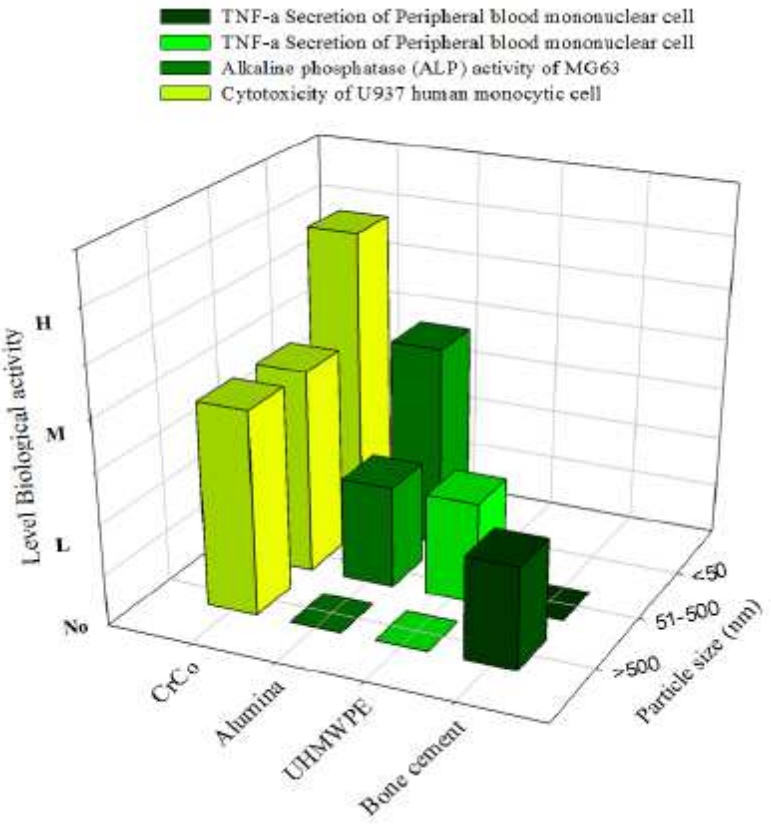
TNF-a Secretion of Peripheral blood mononuclear cell
TNF-a Secretion of Peripheral blood mononuclear cell
Alkaline phosphatase (ALP) activity of MG63
Cytotoxicity of U937 human monocytic cell
Level Biological activity
H
M
L
No
CrCo
Alumina
UHMWPE
Bone cement
<50
51-500
>500
Particle size (nm)

Figure 6

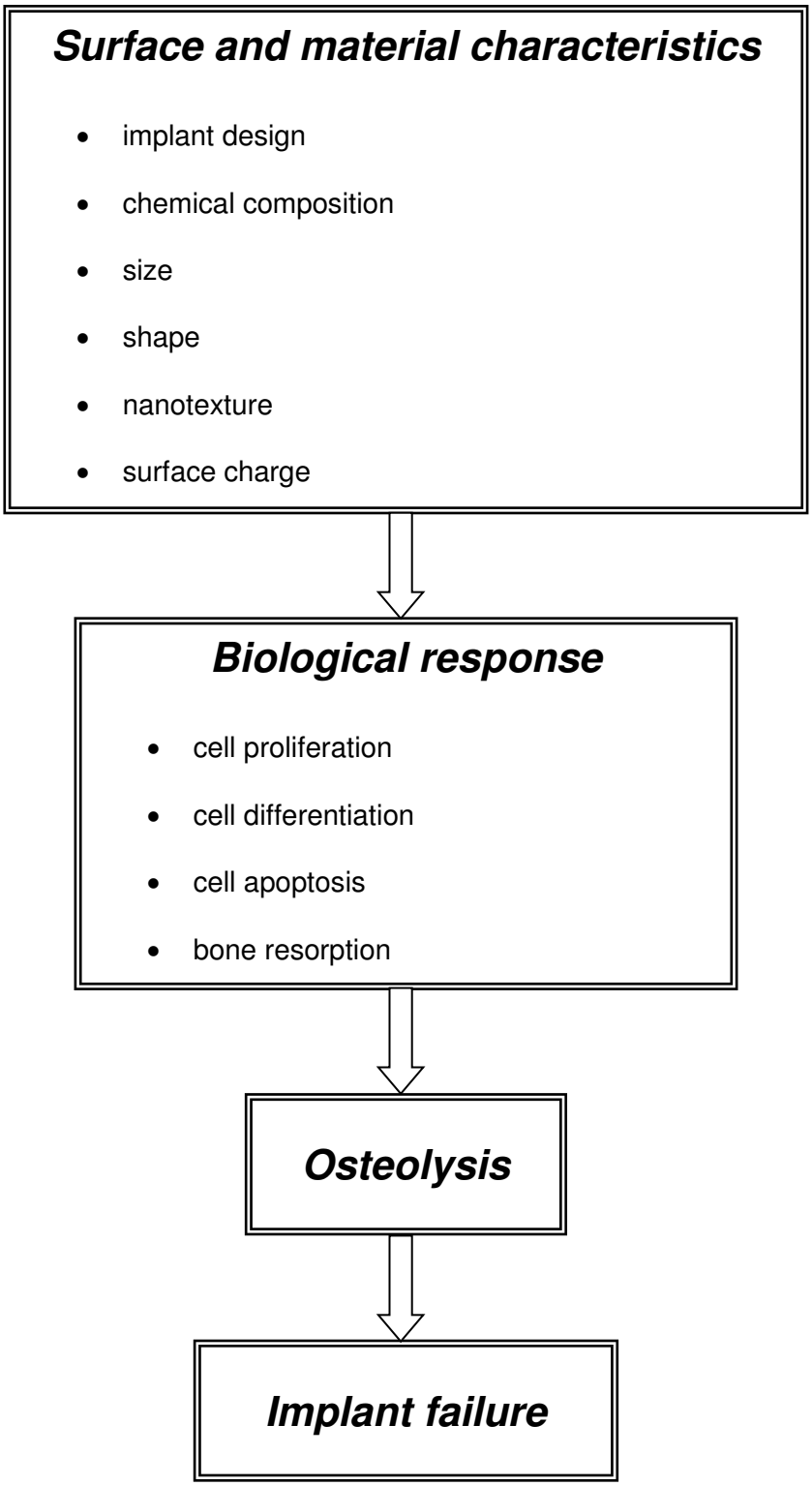
Surface and material characteristics
implant design
chemical composition
size
shape
nanotexture
surface charge
Biological response
cell proliferation
cell differentiation
cell apoptosis
bone resorption
Osteolysis
Implant failure

Figure 7