Institute of Medical &
Biological Engineering

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THE BIOLOGICAL RESPONSE TO NANOMETRE-SIZED PARTICLES

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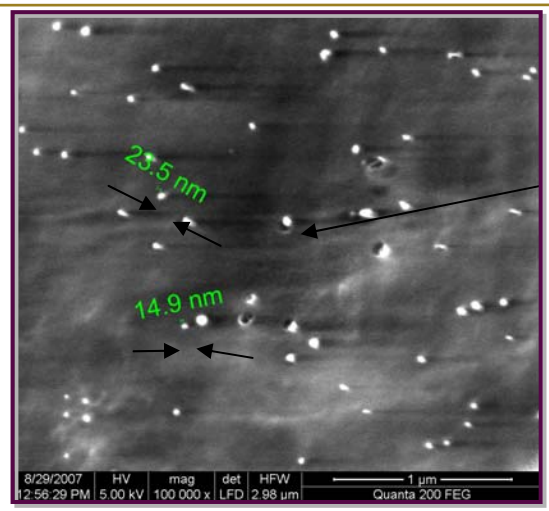
iMBE

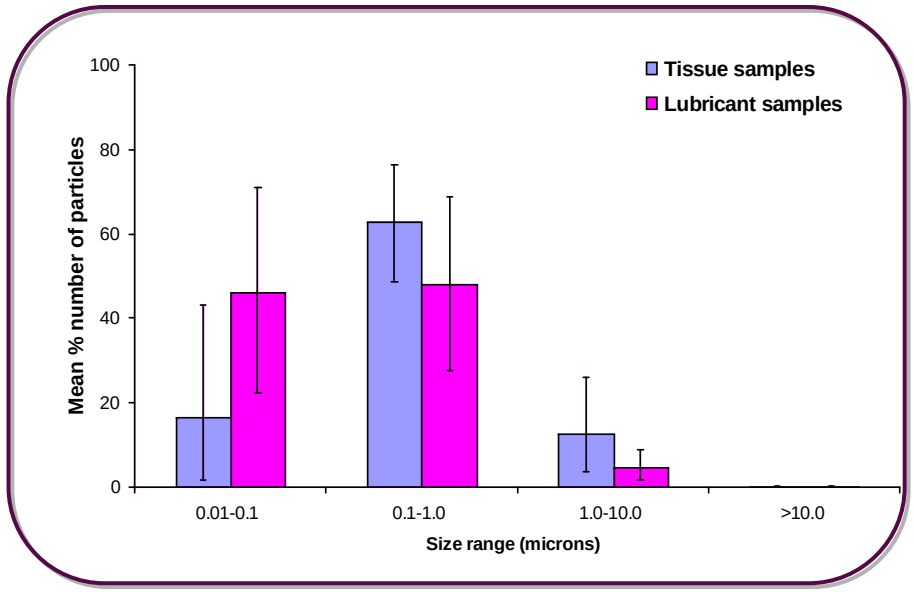
Engineering '50 active years after 50' through multi-disciplinary research, innovation, knowledge creation and translation.

INTRODUCTION

- Access to SEMs with increased resolution is now common.
- Magnifications of >100,000x are achievable.
- Nanometre-sized particles have been isolated from
  - hip[1], knee[1] and spine[2] simulator lubricants.
  - periprosthetic tissues from failed cemented Charnley prostheses[3] and uncemented prostheses[4]
- Highly crosslinked UHMWPE produce more nanoparticles[5]



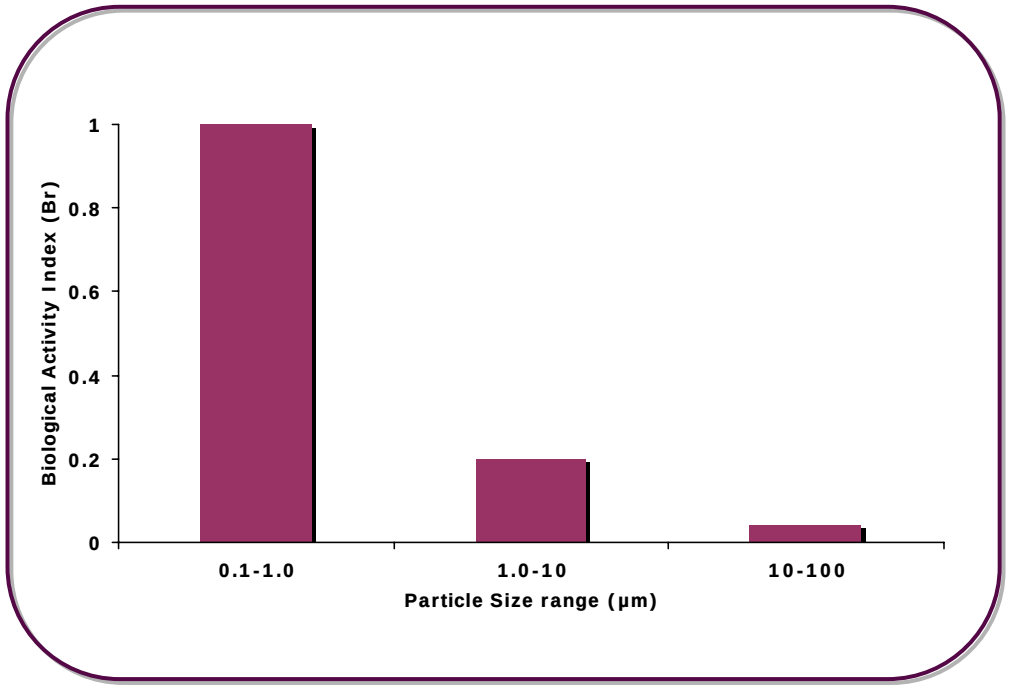
Nanometre sized UHMWPE particle



1. Tipper et al., 2006 JBMR 78A, 473.
2. Tipper et al., 2009 Trans SAS, 107.
3. Richards et al., 2008 JBJS 90B, 1106.
4. Lapcikova et al., 2009 Wear 266, 349.
5. Ingram et al., 2004 Biomaterials 25, 3511.

BIOLOGICAL ACTIVITY OF PARTICLES

- Previously shown that submicron (0.1-1.0 μm) sized particles are the most biologically active[6]
- Osteolytic cytokine release from macrophages
  - TNF-α
  - IL-1β
  - IL-6
  - GM-csf
  - PGE_2
- Biological response to nanoscale particles unknown



6. Matthews et al., 2000. Biomaterials 21, 2033-2044.

HYPOTHESIS

- Nanoscale particles are too small to be taken up by macrophages by the active process of phagocytosis and will be more likely to be taken up by non-active processes such as macropinocytosis. Consequently it is expected that nanoparticles will not stimulate an inflammatory cytokine response from macrophages.

AIMS

- To determine the biological activity of nanometre-sized polymer particles.

- Biological activity was determined by quantification of inflammatory cytokine release from particle stimulated human peripheral blood mononuclear cells.

PARTICLES

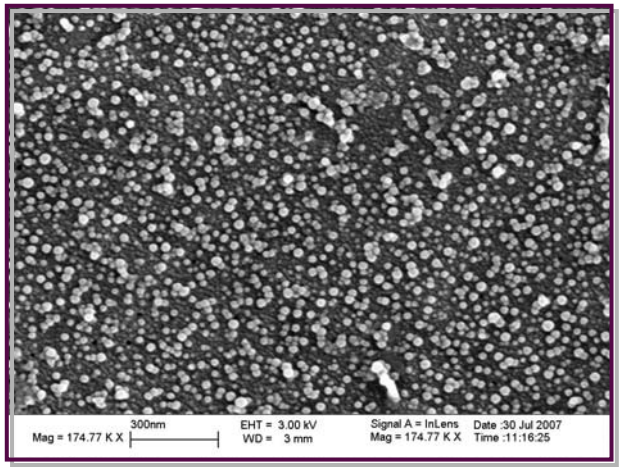
Polystyrene FITC-conjugated FluoSpheres (Invitrogen, UK) in 20 nm, 40 nm, 0.2 μm and 1.0 μm sizes.

GUR 1120 UHMWPE wear debris produced aseptically using a multi-directional wear rig.

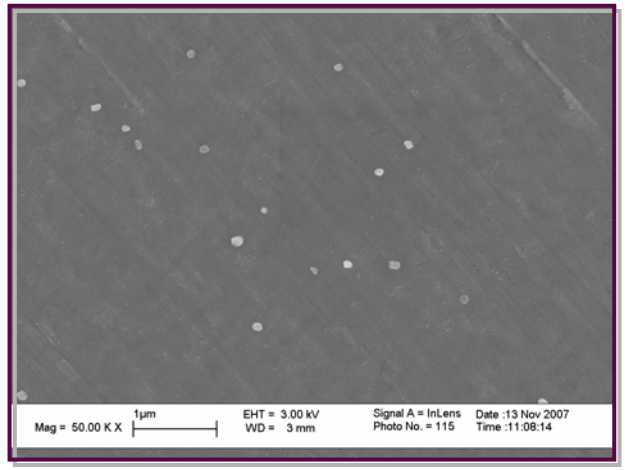
Complete Ceridust® (Hoechst, Germany), a low molecular weight polyethylene powder (size range 15 nm – 53 μm.

Nanometre-sized Ceridust®, fractionated by filtration using 10 μm, 1 μm, 0.1 μm, 0.05 μm, and 0.015 μm filters.

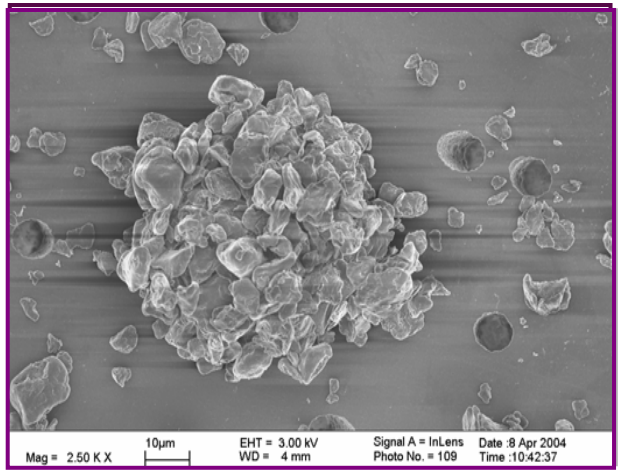
PARTICLES



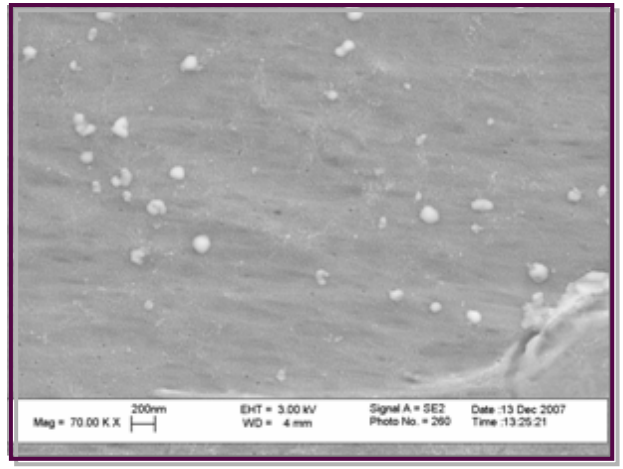
20 nm FluoSpheres



Aseptically generated UHMWPE particles



Ceridust® particles



Nanometre-sized Ceridust® particles

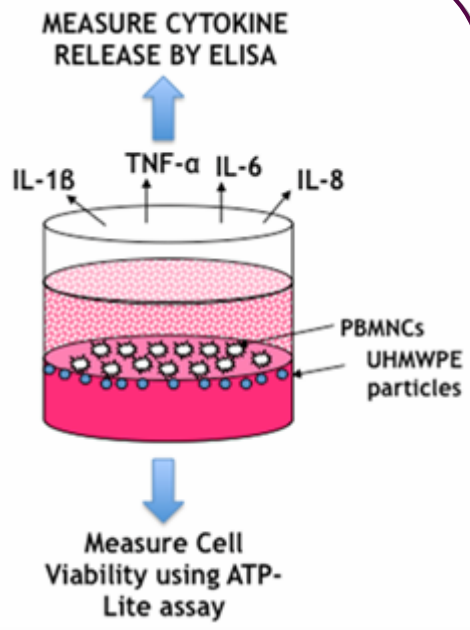
CULTURE OF CELLS WITH PARTICLES

- Human peripheral blood mononuclear cells (3 healthy volunteers)
- Particle volume: cell number = 10 μm^3 per cell and 100 μm^3 per cell

Groups:

1. Cells only negative control
2. Cells + LPS (200 ng/ml) positive control
3. Cells + polystyrene FluoSpheres (20nm, 40 nm, 0.2 µm, 1 µm)
4. Cells + complete Ceridust®
5. Cells + nanometre-sized Ceridust®
6. Cells + aseptically generated UHMWPE wear debris

- 3, 6, 12, 24 h culture
- Cell viability (ATP-Lite assay)
- Osteolytic cytokine release (ELISA; TNF-α, IL-1β, IL-6, IL-8)

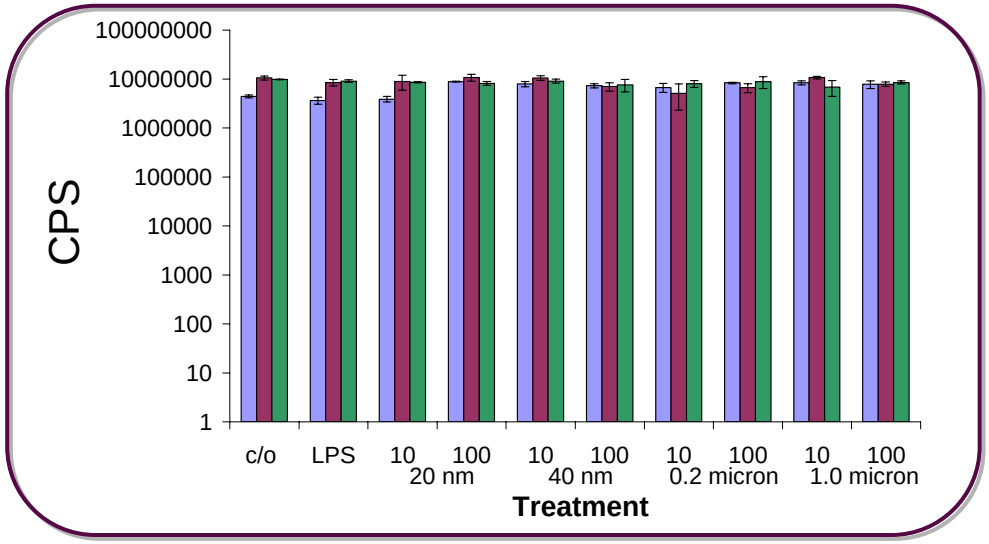


Schematic showing the culture of particles with cells

RESULTS - TNF-α FLUOSPHERES 3h 12h 24h *p<0.05

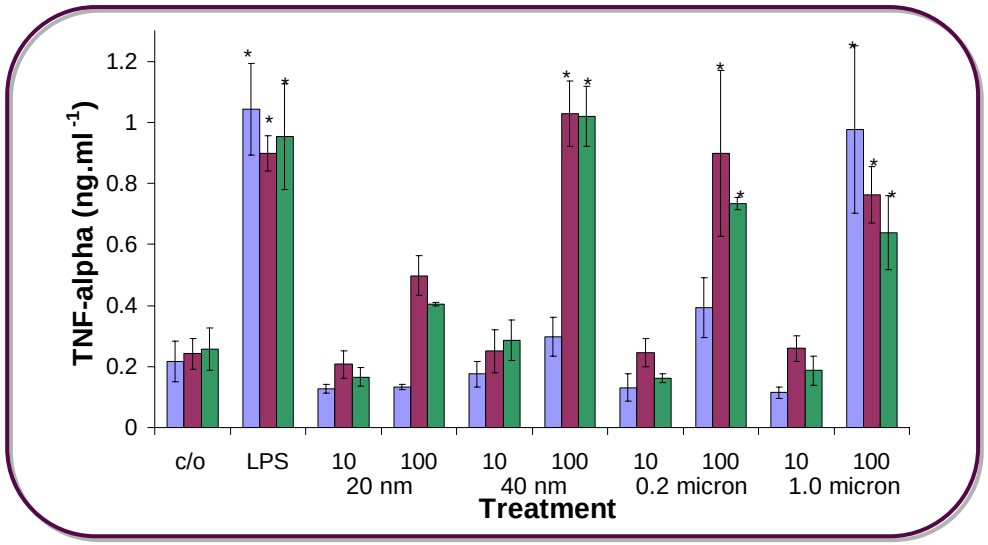
Cell Viability

- No adverse effects on cell viability



Osteolytic Cytokine Release

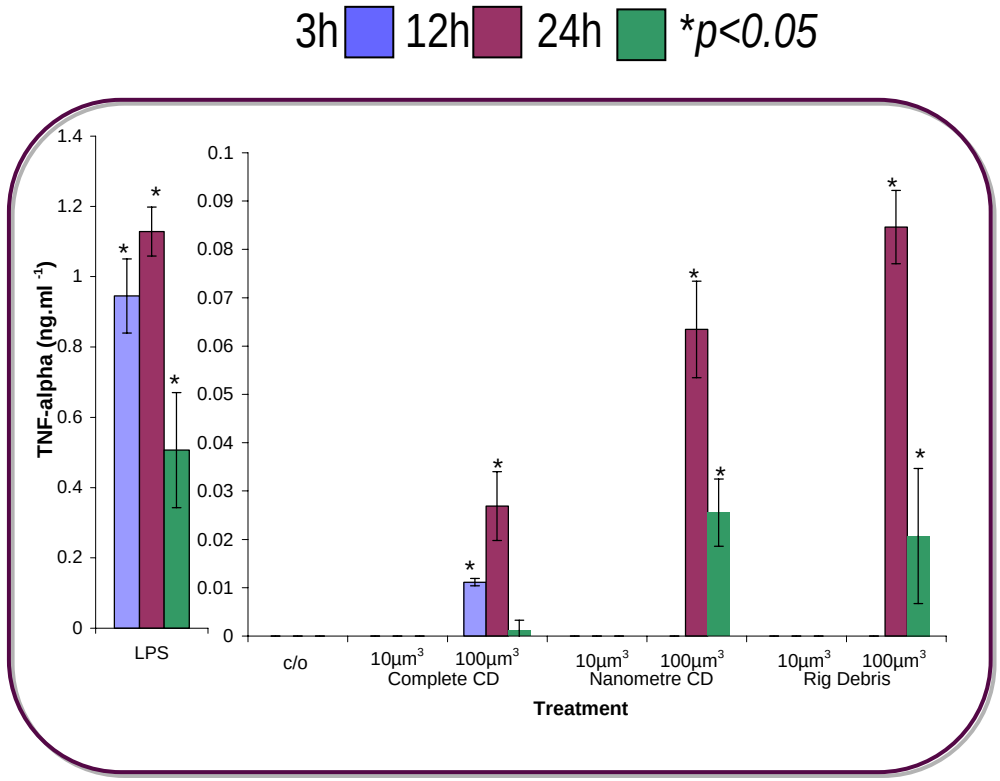
- Significantly higher levels of TNF-α released with particles of 40 nm and above at 12h and 24h with 100 μm^3 particles per cell.
- No response with 20 nm particles



RESULTS - TNF-α PE PARTICLES

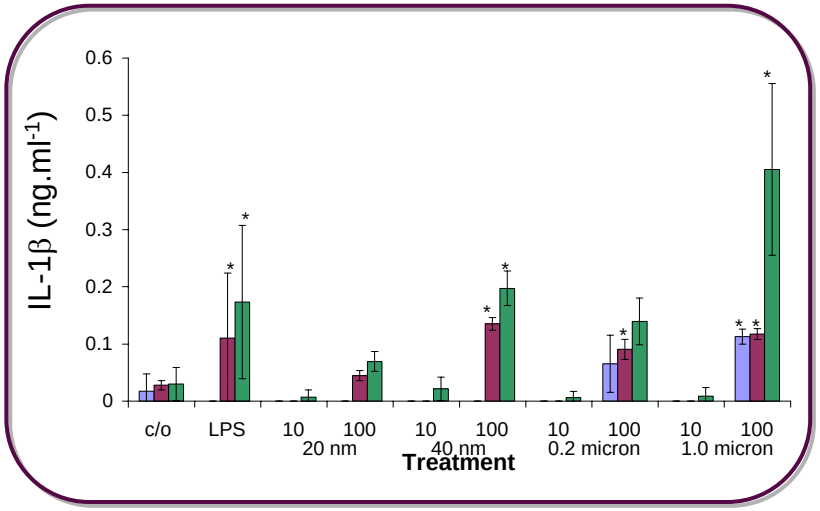
Osteolytic Cytokine Release

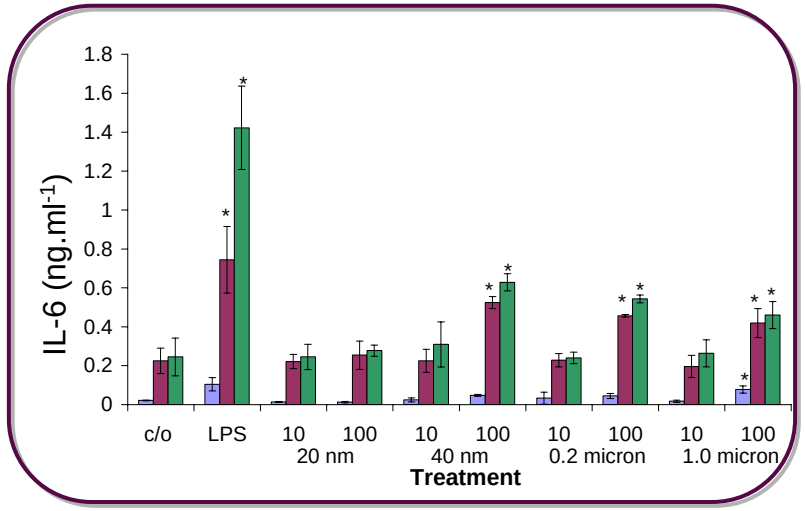
- Significantly higher levels of TNF-α released with nanometre-sized polyethylene particles at 12h and 24h with 100 μm^3 particles per cell.
- Level of response was comparable with total wear volume (as generated)



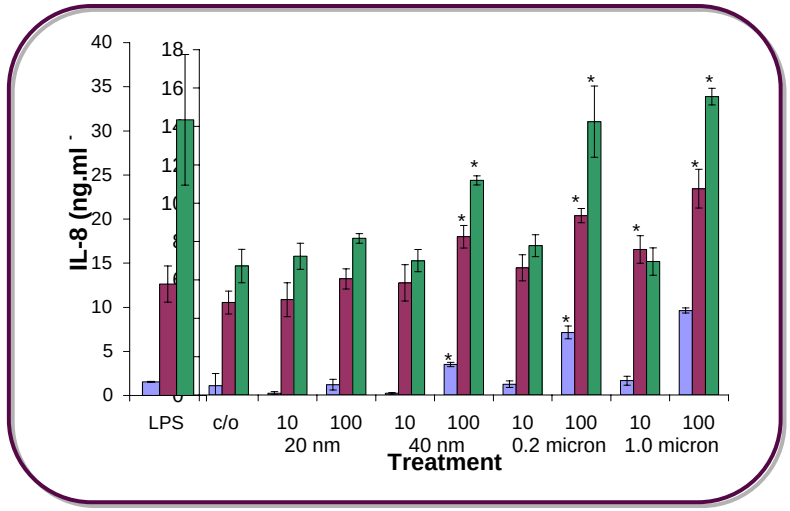
RESULTS - INTERLUEKINS

3h 12h 24h *p<0.05





- Significantly higher levels of interleukins released with particles over 40 nm and above at 12h and 24h with 100 μm^3 particles per cell.
- No response with 20 nm particles



DISCUSSION

- Nanometre-sized particles have the potential to provoke inflammatory cytokine release from macrophages.
- There was a lower particle size limit, with the 20 nm FluoSpheres showing the lowest activity.
- Nanometre-sized polyethylene particles (Ceridust®) caused elevated TNF-α release at 100 μm^3 per cell from cells from 3/3 donors at 12 hours and from 2/3 of the donors at 24 hours.

FUTURE WORK

- Generation of aseptic nanometre-sized UHMWPE particles by articulation in POP rig.
- Fractionation of UHMWPE wear debris to isolate nanometre-sized UHMWPE wear debris
- Determine inflammatory cytokine response to nanometre-sized UHMWPE wear particles
- Investigation into the uptake of nanometre-sized wear debris. Are these particles taken up by mechanisms other than phagocytosis (eg macro/micro pinocytosis)?

ACKNOWLEDGEMENTS

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DePuy International

arc 0870 850 5000 www.arc.org.uk Committed to curing arthritis

EPSRC Engineering and Physical Sciences Research Council