

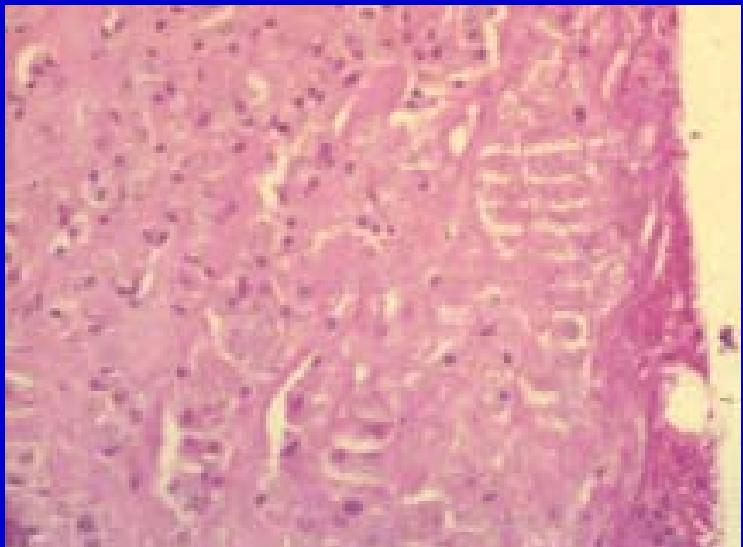
The clinical problem: Implants for Joint replacements generate wear particles of different size distributions

Cytokines of macrophages after phagocytosis of nano- or micro-sized corundum, graphite and chromium oxide

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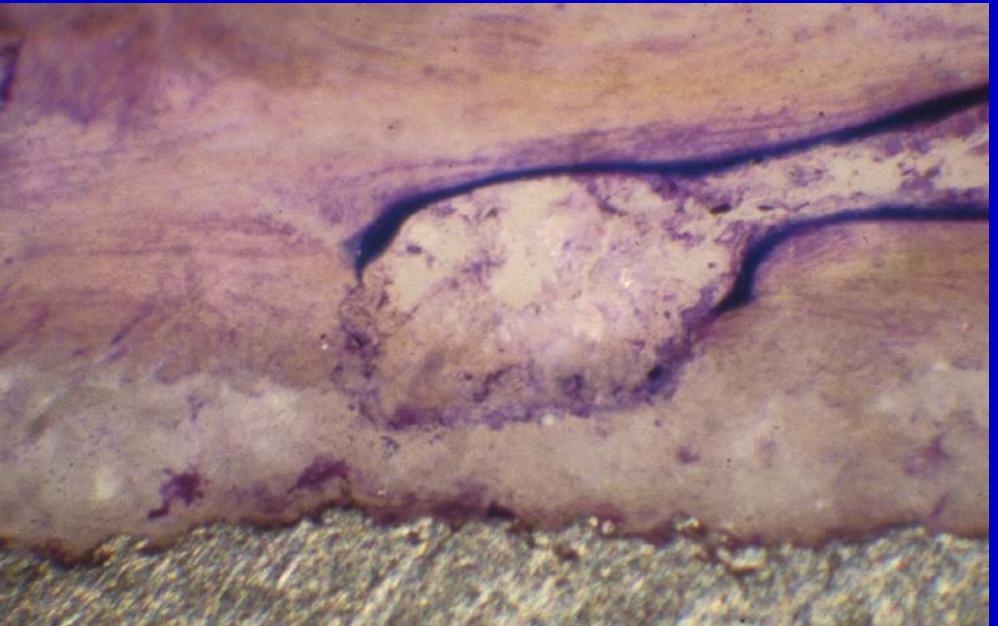
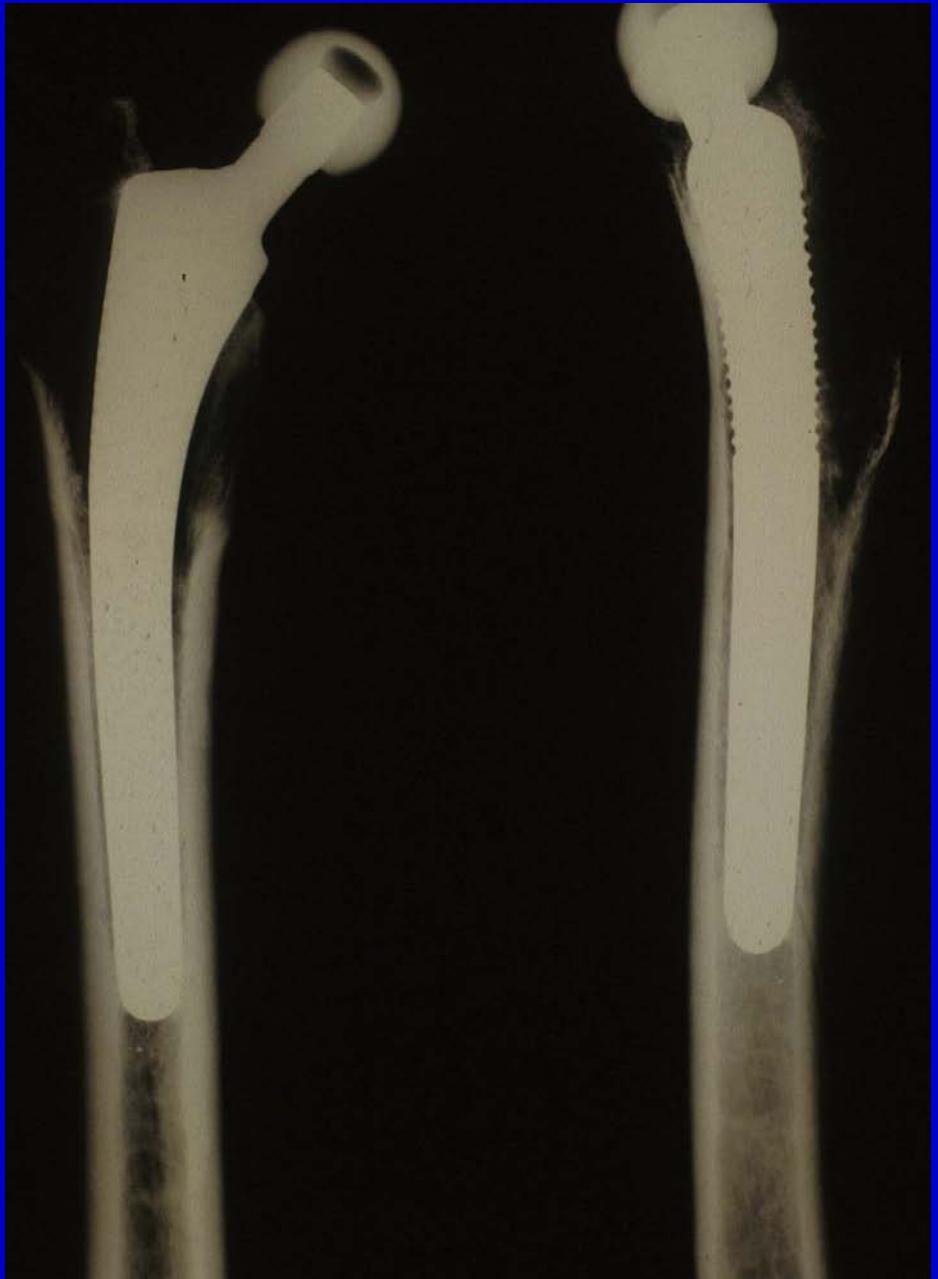
¹Bundesanstalt für Materialprüfung und -Forschung, Unter den Eichen 44-46,
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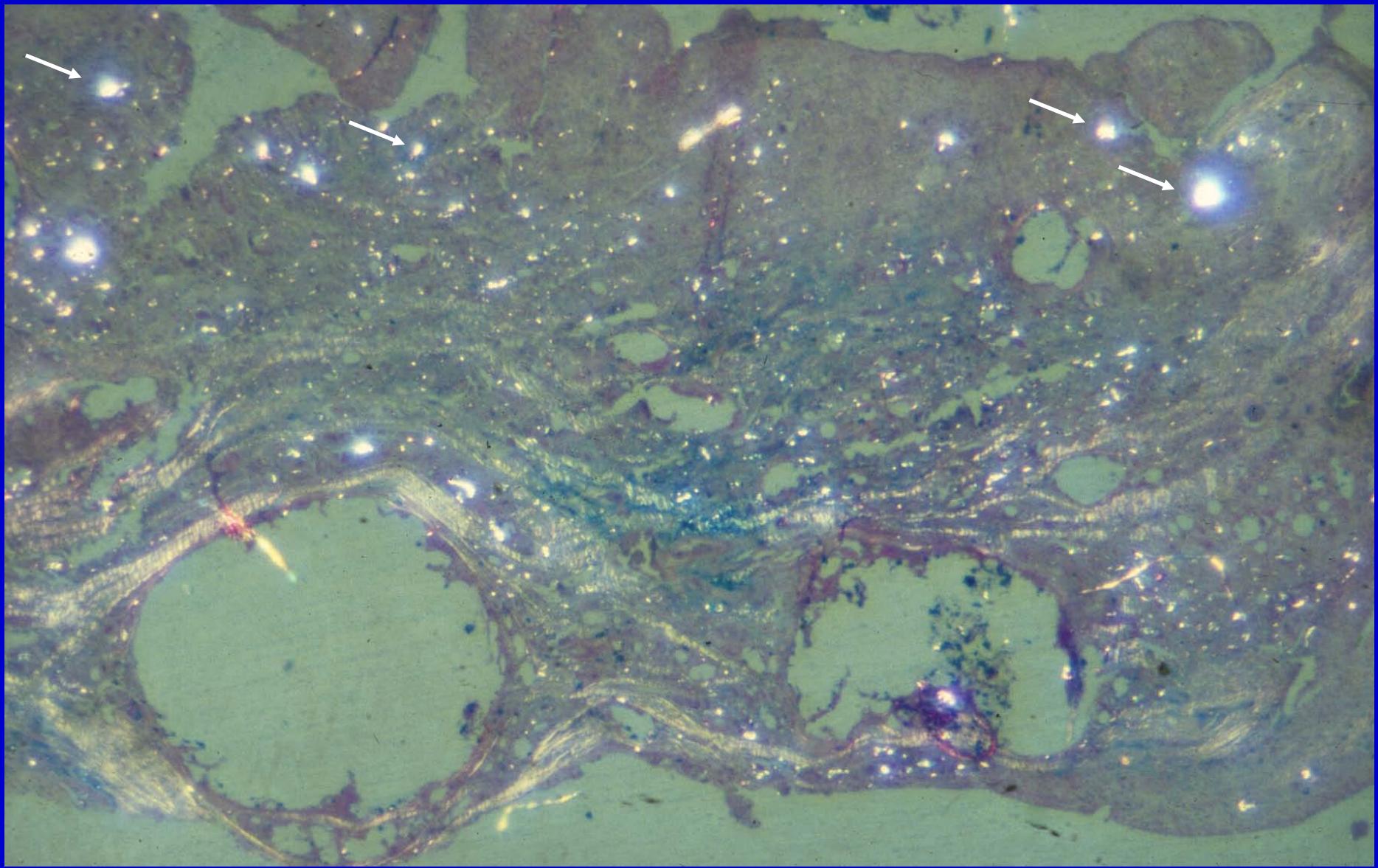


Cemented metal / metal total hip prosthesis after 25 years.

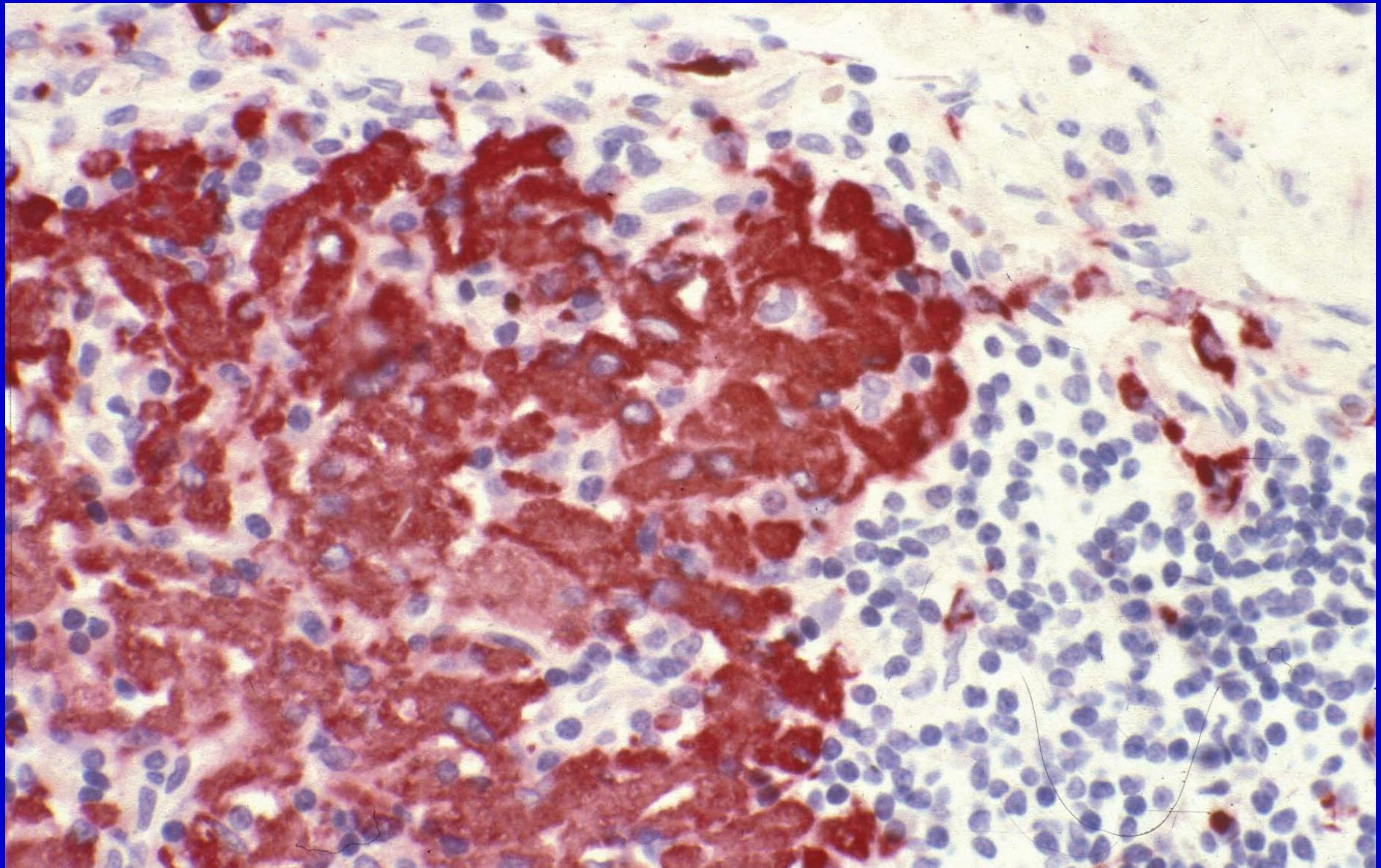
superficial necrosis of the neo-synovia
with macrophages full of small
particles. H&E.



Hydroxyapatite coated CoCrMo-alloy
stem, Al_2O_3 ceramic head,
one year after implantation in the
left femur, Male, 125 kg BW,
E 13421-92



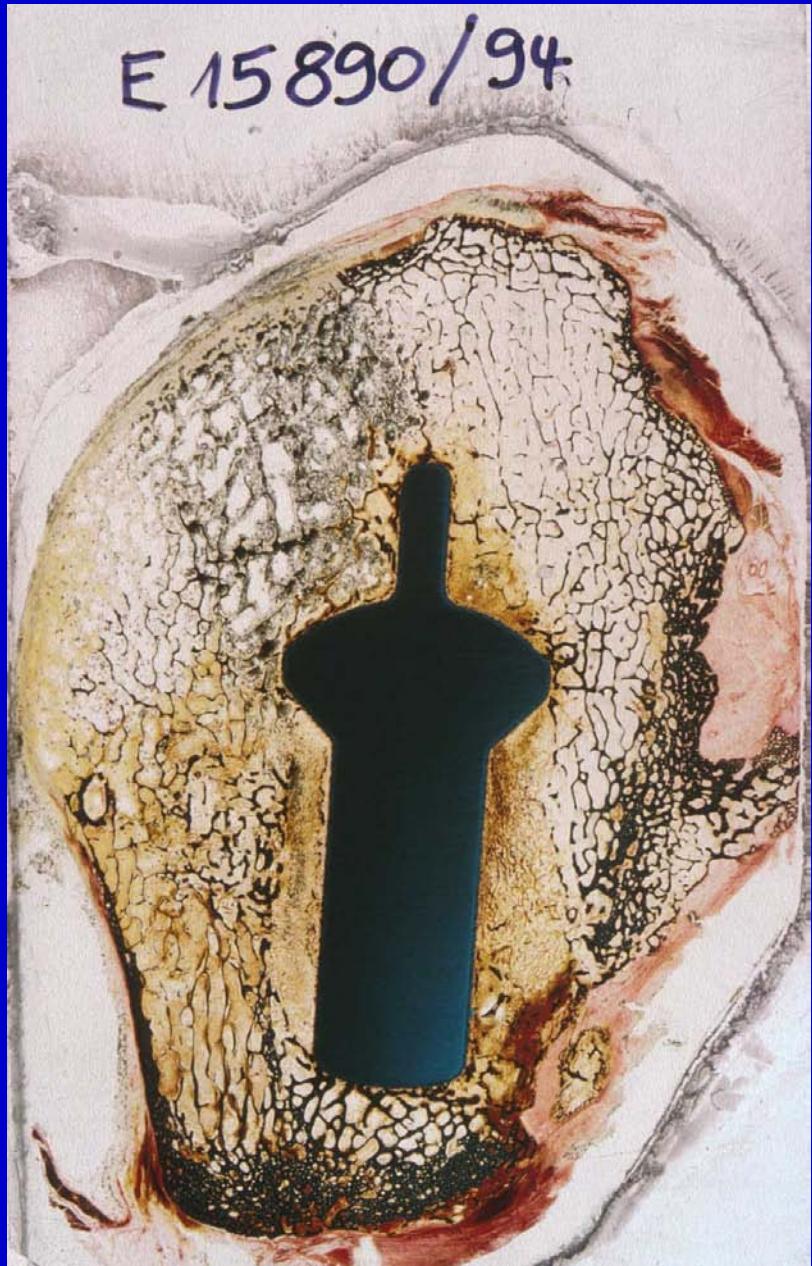
left humero-acromial joint replacement 2 years, change of the prosthesis
3 months ago. Female 80 years. E 5148-80, polarized Nicols prisms.



IH PGM1 macrophages, E 4444-91

	Fe	Cr	Ni	Co
n	102	70	31	50
max	5629	2646	739	2746
min	101	0.7	3	4
mean	936	197	64	239

Content of Fe, Cr, Ni and Co in mg/kg or ppm in tissue adjacent to failed, surgically removed hip and knee prostheses, 79 cases. n = number of positive determinations



Plasmapore coated
Aesculap BiContact® stem

Femur



Background

**Aseptic loosening of endoprostheses
is mainly correlated to generation of particles**

**Particles are released from gliding surfaces of metal, metal alloy,
UHMWPE, Alumina Al_2O_3 ,
bone cement and components, e.g. zirconium**

The size of particles varies from nm to μm

**Particles are found in the interface, around the interface,
adjacent tissues, lymph nodes, lung, spleen, liver and other organs**

**Particles are phagocytosed by macrophages and
antigen presenting cells**

Stimulation of cytokines in murine macrophages by micro- and nano-sized particles

Macrophages

produce cytokines

e.g. IL-1 β , IL-6, IL-10, IL-12, G-CSF,
GM-CSF, TNF- α , KC,
nitrogen oxide (NOx).

**influence T-cell and B-cell response
i.e. the immune response**

**sensitive against Lipopolysaccharide (LPS)
derived from the external part of the wall of
Gram- negative bacteriae**

Lipid A anchored in the bacterial wall

Objectives

Aluminium oxide polycrystalline ceramics as gliding components for hip and knee endoprostheses generate the smallest number of particles of nm and μm size.

From new very thin coatings of diamond-like carbon to metallic modular endoprostheses nm size carbon particles could be released.

Co-Cr-Mo alloys are used and chromium containing particles could be released.

Question:

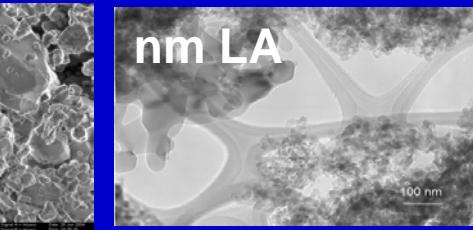
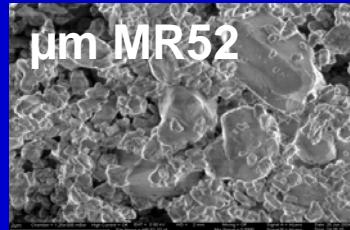
Do particles of nm or μm size generate different cytokines and NO_x ?

Materials and Methods

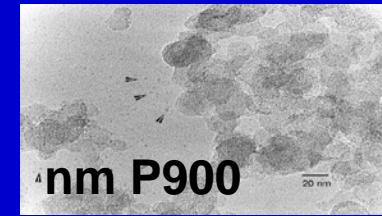
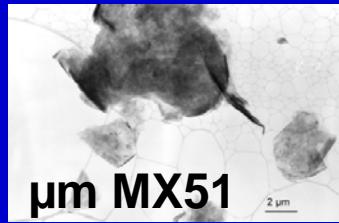
Characterization of materials

(Wear 264 (2008) 505-517

JBMR-A Published Online: Apr 22 2008 ISSN: 1552-4965)

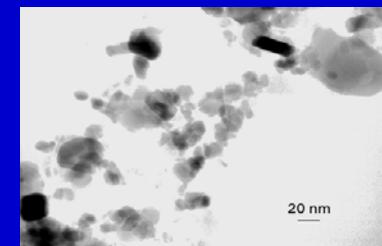


corundum



graphite

chromium (III) oxide

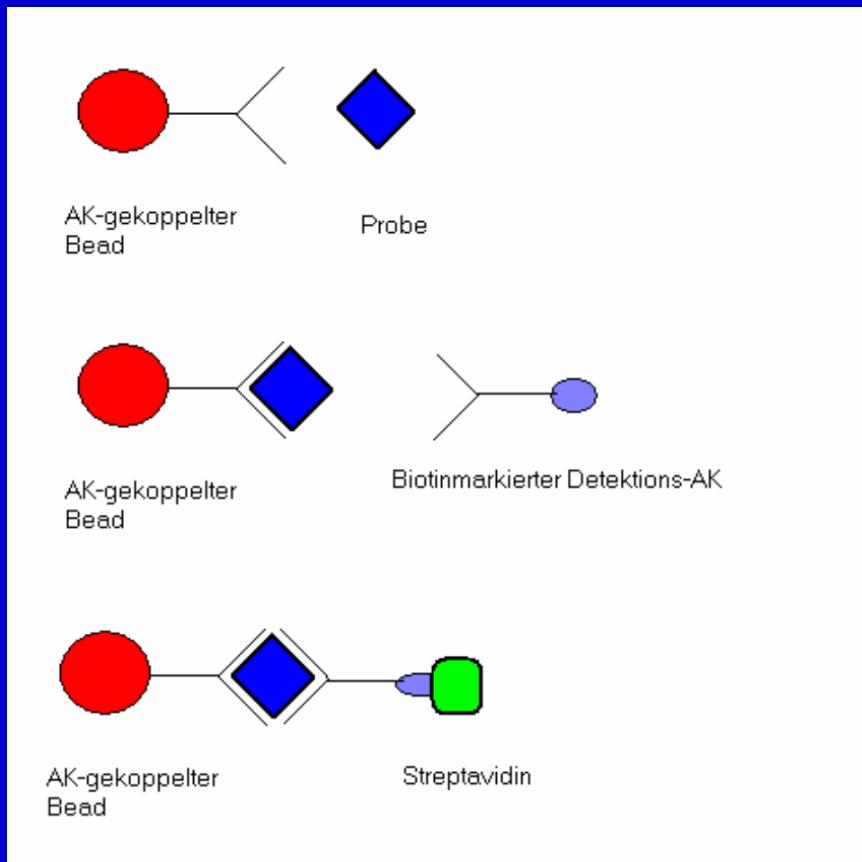


Murine macrophages (RAW264.7 cells) ASTM standard F 1903

- Macrophages morphology, vitality, SEM, TEM
- Cytokines by Multiplex- ELISA
- NOx by Griess reagent
- Statistics ANOVA Man-Whitney test
- Gene expression profiles (mRNA) by TaqMan-PCR

particles	density [g/cm ³]	Vol- conc. [Vol.-%]	40 ml sus- pension (0,8 ml solid)	D ₅₀ [µm]	Zetapotential [mV] / pH			
					Cell culture medium		Dist. water	
					Zetapot.	pH	Zetapot.	pH
corundum MR 52 (µm)	3,9442	2	3,19 g	1,7	+7,09	8,68	-14,16	8,68
corundum LA (nm)	3,6768	2	2,94 g	0,04	+3,17	8,48	+7,56	7,79
Cr ₂ O ₃ (nm)	5,0587	1	2,025	0,1	+0,66	8,0	-1,21	4,46
graphite MX 51 (µm)	2,2735	2	1,82 g	6,3	+25,29	8,25	-10,4	10,33
graphite (nm) Printex 90C	2,0380	1	0,815 g	0,1	-9,49	8,0	-3,25	8,6

Cytokine determination by Multiplex-ELISA (Bio-Plex, BioRad)



1. incubate antibody-coupled beads with sample or standard

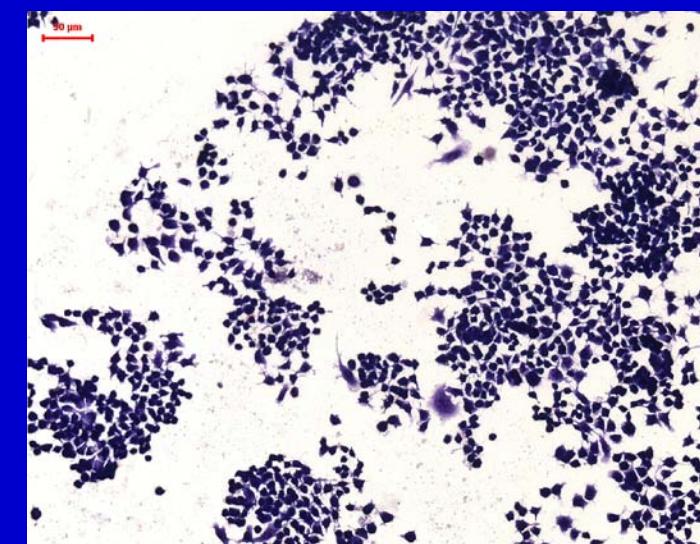
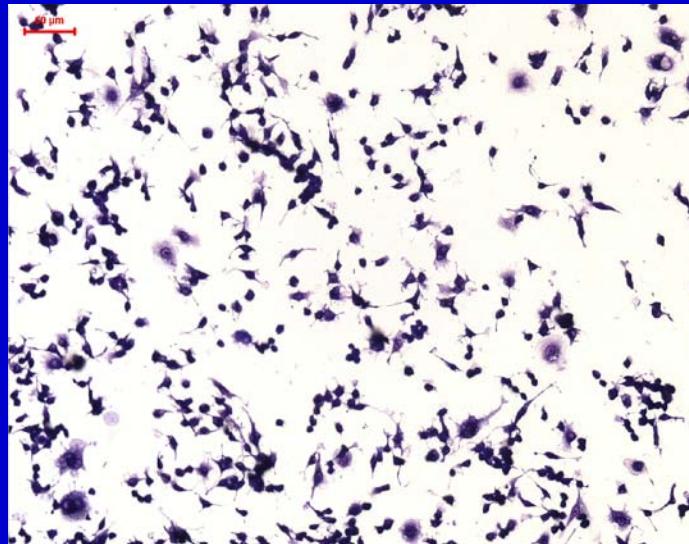
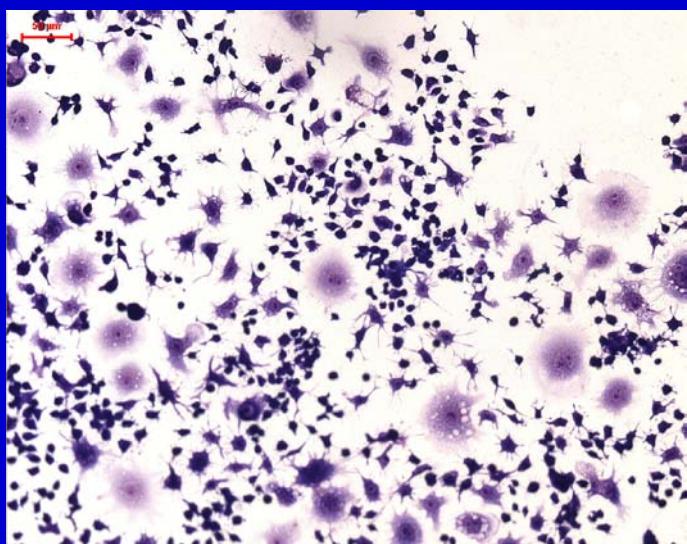
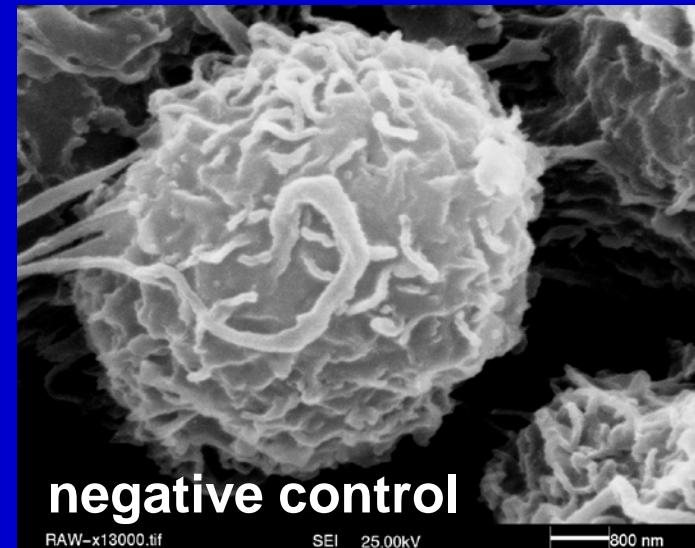
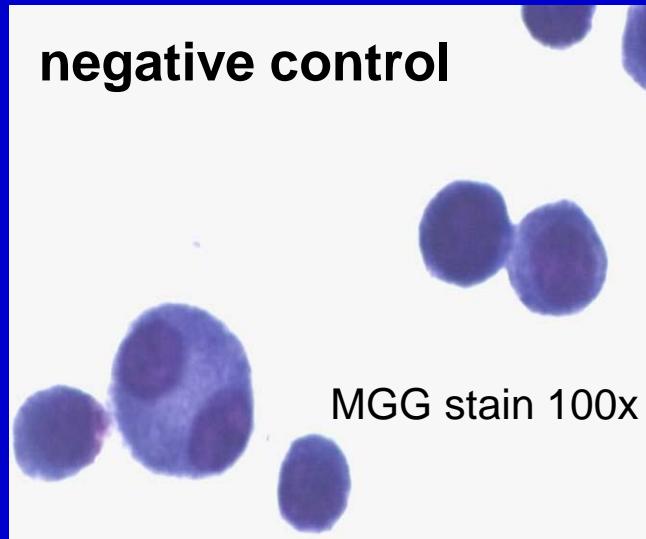
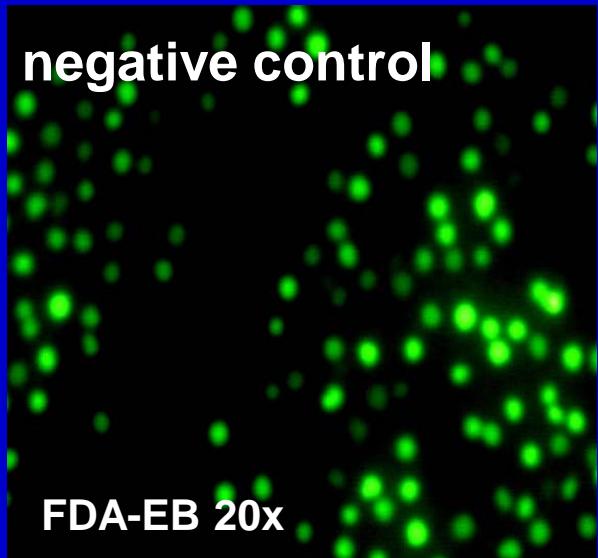
2. wash, then incubate with biotinylated detection antibody

3. wash, then incubate with streptavidin-PE; read on the Bio-Plex suspension array system

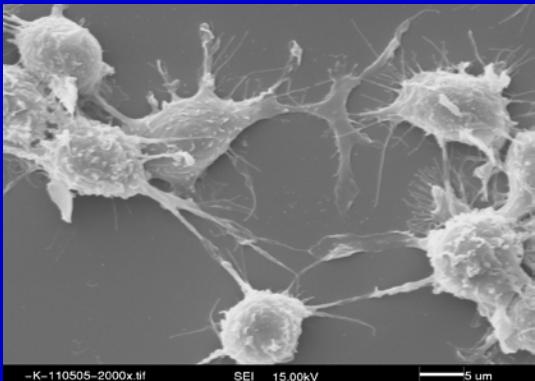
**IL-1 β , IL-6, IL-10, IL-12 (p40), IL-12 (p70),
TNF-a, KC, G-CSF, GM-CSF**

Results

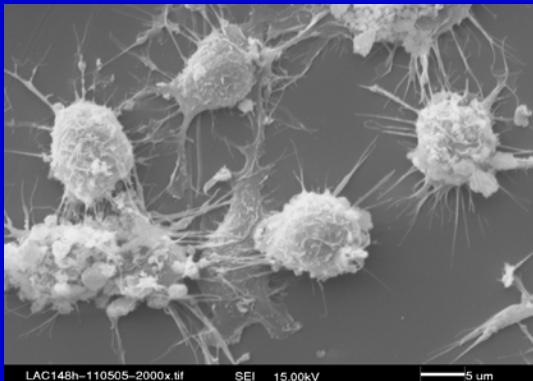
cells RAW264.7, corundum



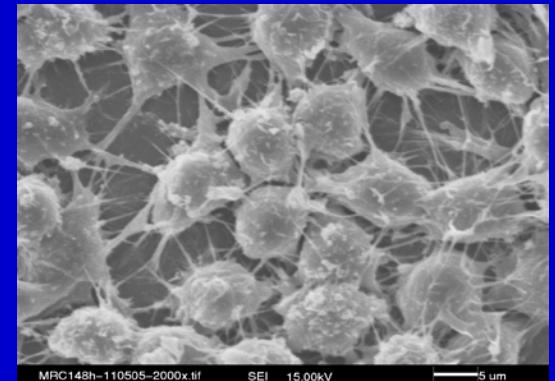
SEM (RAW264.7)



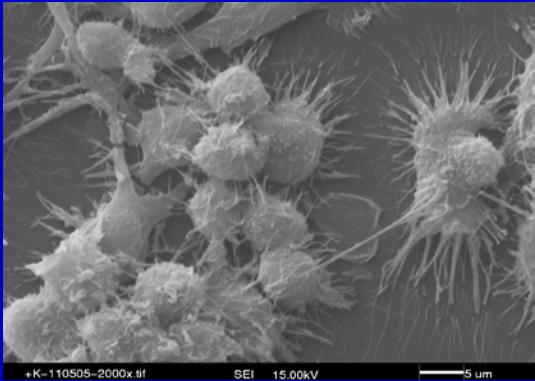
-C, 48h, 2000x



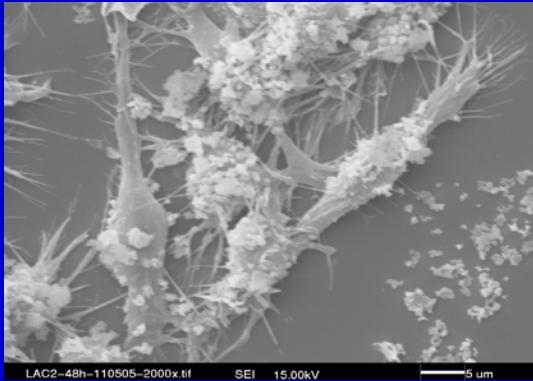
La c1, 48h, 2000x



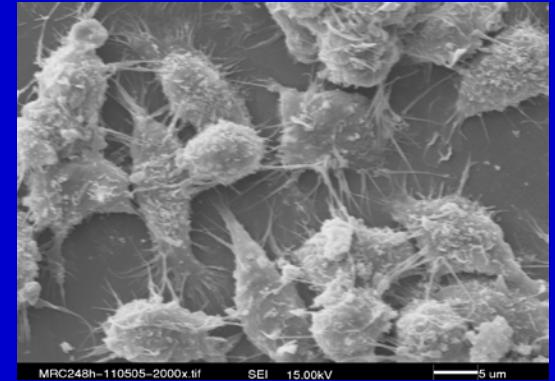
Mr c1 48h, 2000x



+C, 48h, 2000x

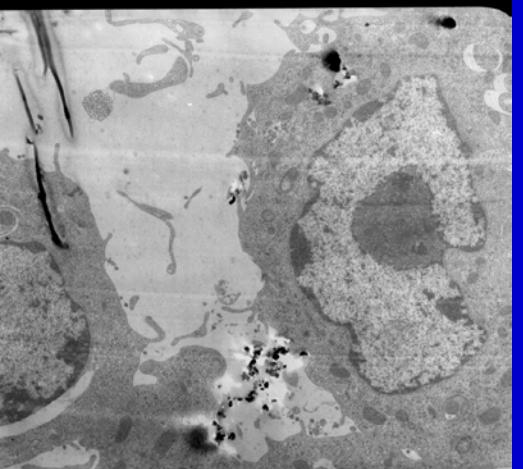


La c2, 48h, 2000x



Mr c2, 48h, 2000x

KT 001675 12E-10 M7000 <-->1.1 μ m
RAW LAC1/48H U80-0000 I12E-10

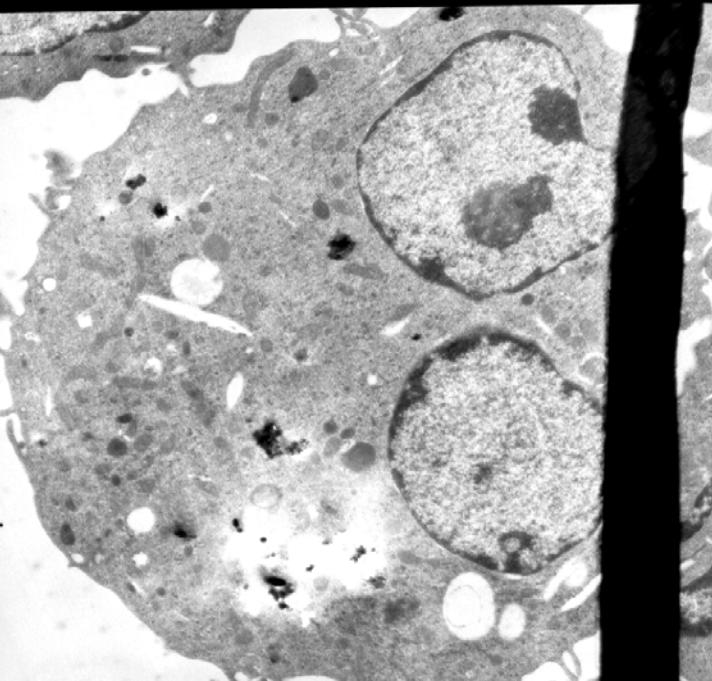


TEM

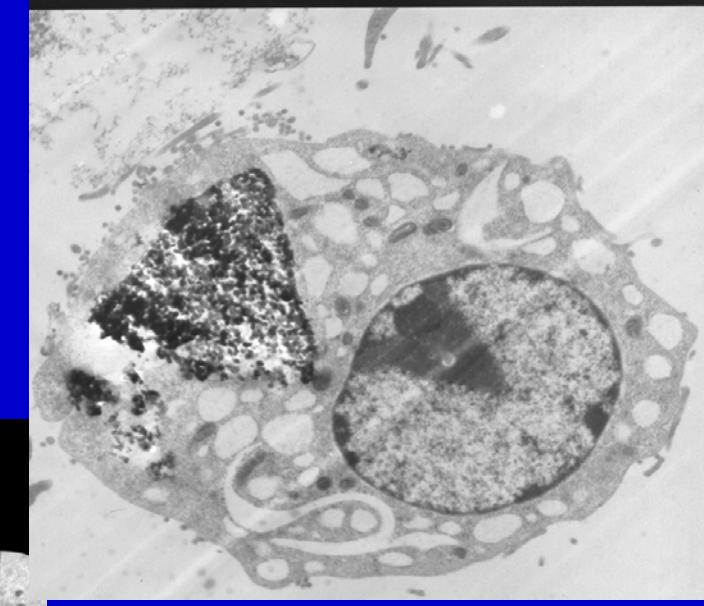
nm
particles

KT 001674 12E-10 M20000 <--> .4 μ m
RAW LAC1/48H U80-0000 I13E-10

KT 001678 050607 M7000 <-->1.1 μ m
RAW LAC1/96H U50-0000 I12E-10

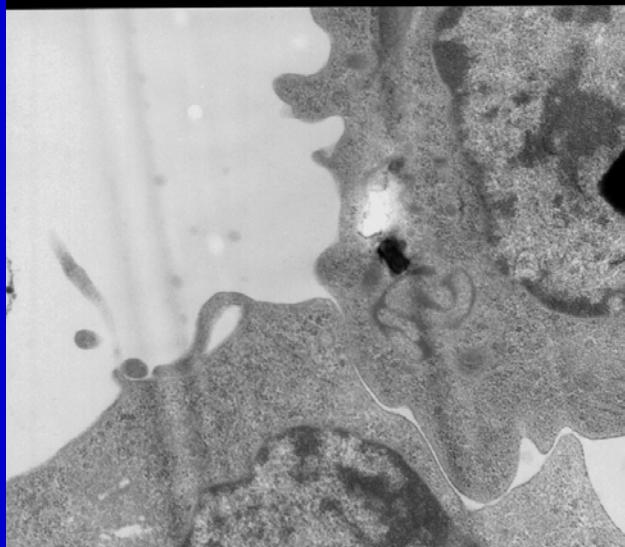


KT 001691 050607 M7000 <-->1.1 μ m
RAW LA/C148H U50-0000 I12E-10



attachment
internalization
phagocytotic vacuoles
secondary lysosomes
vacuoles as sign of
stress

KT 001677 12E-10 M12000 <--> .6 μ m
RAW MRC1/48H U80-0000 I12E-10



KT 001685 050607 M12000 <--> .6 μ m
RAW MRC1/96H U50-0000 I12E-10

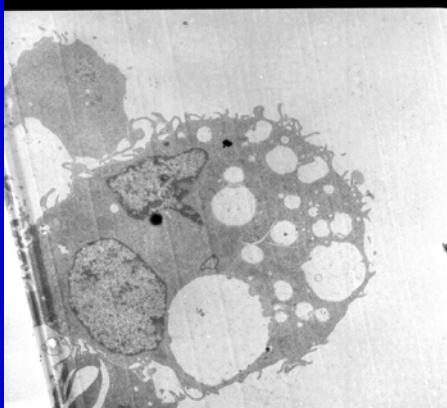


TEM
 μ m particles

KT 001689 050607 M20000 <--> .4 μ m
RAW MR/C148H U50-0000 I55E-11



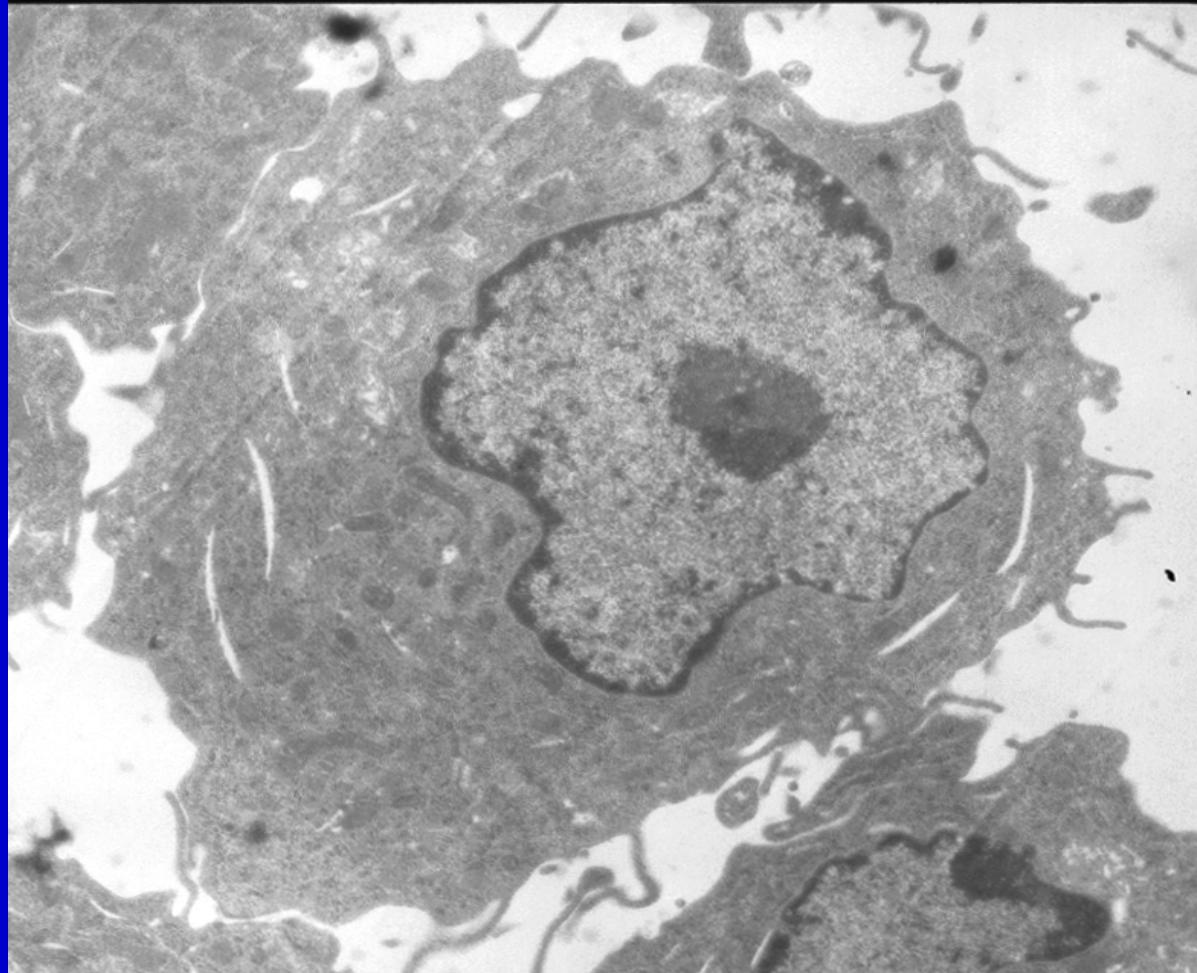
KT 001671 12E-10 M3000 <-->2.5 μ m
RAW+K48H U50-0000 I12E-10



+ and -
controls

KT 001680 050607 M7000 <-->1.1 μ m
RAW -K /96H U50-0000 I12E-10

KT 001680 050607 M7000 <-->1.1 μ m
RAW -K /96H U50-0000 I12E-10



Gene profiling of 48 murine mRNAs

by TaqMan-Low-Density-Array

CDK2 (cyclin-dependent kinase 2)

GAPDH

HPRT

Bax

Bcl2

Caspase3

Caspase9

iNOS

CDw136

CD206

(FCeRI)

CD14

CD16(FCgRIII)

C5a

CD68

CD32 (FCgRII)

CD35 (CR2)

CD64 (FCgRI)

CD170

SR-A (macrophage scavenger r I)

HIP1

Dynamin 1

Dynamin 2

G-CSF

GM-CSF

IL-10

IL-1b

IL-6

IL-12(p40)

MCP-1

Prostaglandin E Synthase 2

TNF alpha

MIP-1a

IL15

M-CSF

IL1-Rezpetor

IL-1-receptor associated kinase1

TNF Receptor 1A (receptor 1)

TNF Receptor 1B (Receptor 2)

TRAF6

Ubiquitin

Rho GTPase (activating prot. 1)

Rho GTPase (activating prot. 4)

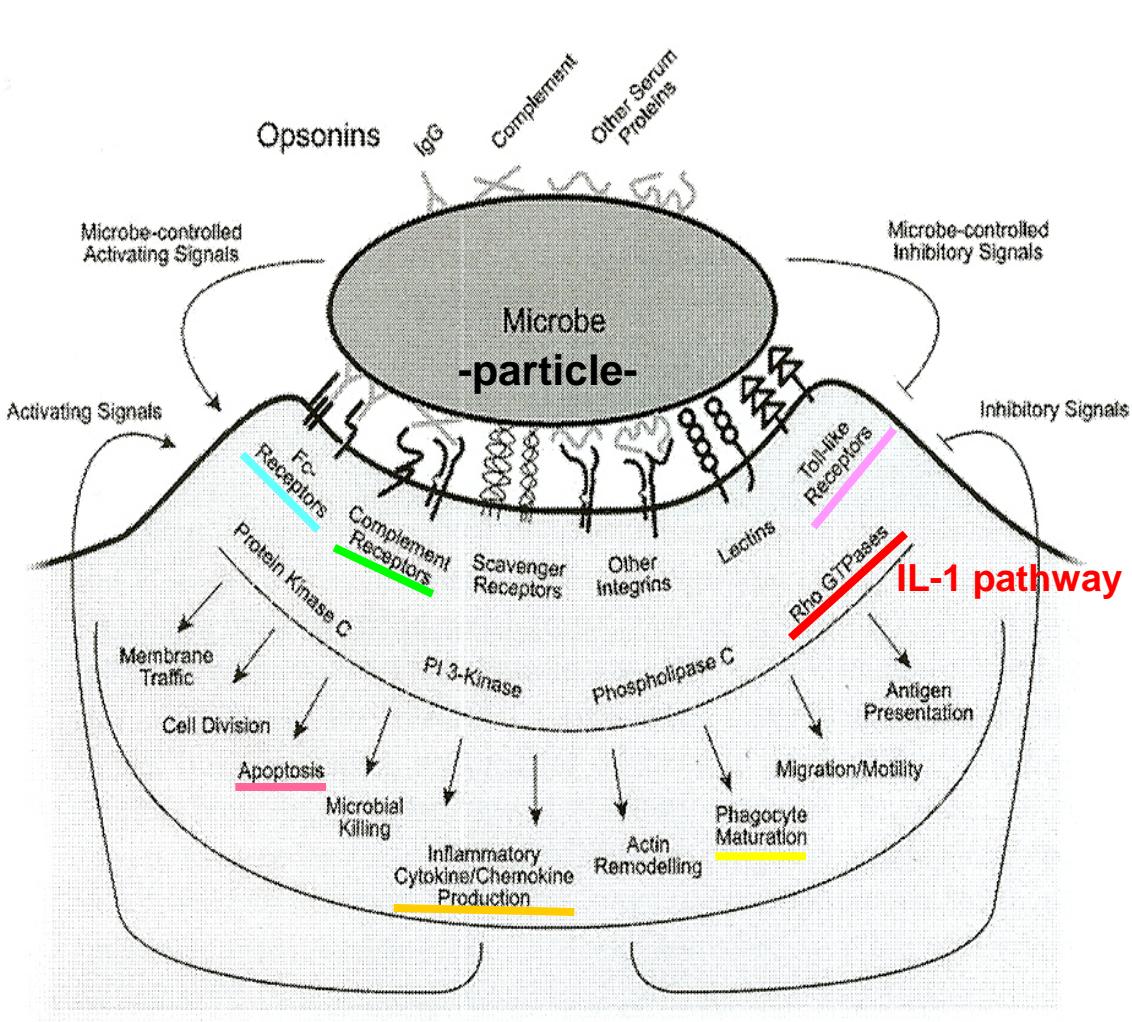
Rho GTPase (activating prot. 26)

Rho GTPase (activating prot. 29)

Cathepsin H

TLR4

NF kappa B1



**Receptor and signaling interactions
during phagocytosis of microbes/particles**

(modified from D.M.Underhill and A.Ozinsky, Annu.Rev.Immunol.2002.20:825-852)

Differentially regulated murine mRNAs after incubation of macrophages with different materials (24-72h incubation)



Nr.	Gene Symbol	Gene name	Receptor and signalling interactions	Nr. of tested materials influencing gene expression
1	Csf1	M-CSF	Cytokine	11
2	Nos2	iNOS	Oxidative Stress	10
3	Arhgap1	Rho GTPase (ac)	IL-1 pathway	10
4	Csf3	G-CSF	Cytokine	10
5	Cr2	CD35 (CR2)	Complement	10
6	Dnm1	Dynamin 1	Complement	10
7	Mrc1	CD206	Phagocytose	9
8	Il12b	IL-12(p40)	Cytokine	9
9	Arhgap4	Rho GTPase (ac)	IL-1 pathway	8
10	Arhgap26	Rho GTPase (ac)	IL-1 pathway	7
11	Il10	IL-10	Cytokine	7
12	Il1r1	IL1-Receptor	IL-1 pathway	6
13	Uchl1	Ubiquitin	IL-1 pathway	6
14	Ccl3	MIP-1a	Cytokine	6
15	Il1b	Il-1b	Cytokine	6
16	Fcgr3a	CD16(FCgRIII)	Phagocytose	5
17	Msr1	SR-A (macropha)	Phagocytose	5
18	Ctsh	Cathepsin H	IL-1 pathway	5
19	Tnfrsf1b	TNF Receptor 1b	IL-1 pathway	5
20	Traf6	TRAF6	IL-1 pathway	5
21	Cd68	CD68	Fc-Receptors	5
22	Il15	IL15	Cytokine	5
23	Dnm2	Dynamin 2	Complement	5
24	Bax	Bax	Apoptose	5
25	Casp9	Caspase9	Apoptose	5

Quantification of differential gene expression of 48 murine mRNAs by TaqMan-LDA

Levels of different protein concentration and mRNA level of selected cytokines

– μm - and nm-sized corundum after 72h incubation

TNF- α

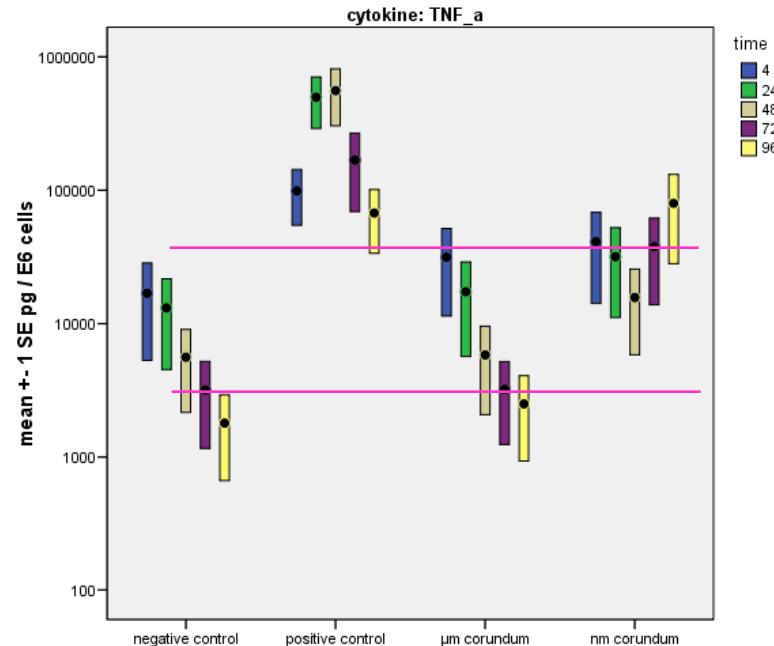
G-CSF

Delta Ct (PCR cycles) vs. Housekeeping gene CDK2

		Csf2	Il10	Dnm1	Arhgap4	Ccl2	Arhgap26	Ccl3	Tnf	Uchl1	Il12b	Csf3	Mrc1	Csf1	Cr2	Il1r1	Mst1r	Arhgap1	Il1b	Nos2	
24h	NC		-4.95	-0.40	-3.51	-0.17	0.79	-1.66	-2.26	0.12	3.44	-0.78	3.02	-9.07	-0.85	5.25	4.96	7.77	-0.42	-1.16	-2.49
24h	Corundum	M52- μm	-1.40	1.39	-1.11	0.77	2.74	-0.95	0.58	1.02	3.42	0.04	-1.49	-8.07	1.25	6.69	6.09	0.40	0.40	-0.48	-1.26
24h		LA-nm	-3.24	1.89	-0.18	0.29	3.19	0.64	1.02	1.29	4.22	0.45	4.54	-6.80	2.22	6.85	1.92	5.82	2.15	-0.64	-1.12
72h	NC		-0.45	5.38	4.15	3.41	-1.49	8.80	0.91	-1.78	3.82	18.19	12.77	10.39	11.30	19.06	11.02	11.77	17.45	6.44	6.92
72h	Corundum	M52- μm	2.58	4.55	-0.18	-1.38	3.61	0.69	3.01	1.83	2.08	1.57	9.26	2.20	5.10	10.51	3.15	0.74	-0.05	7.28	7.72
72h		LA-nm	1.94	0.95	4.63	5.05	-3.75	8.28	-2.27	-3.99	1.43	17.70	-3.44	11.51	7.15	-1.90	8.73	10.93	19.16	0.29	-0.35

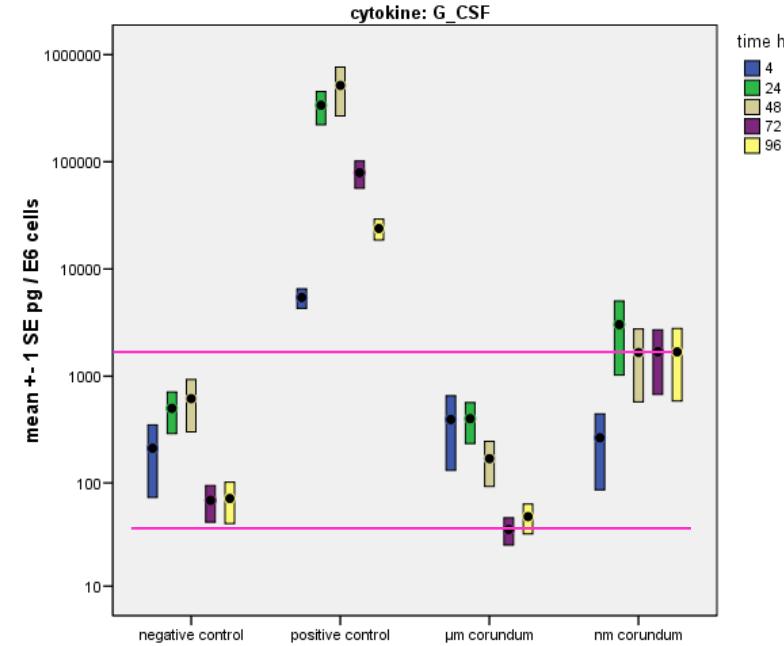
TNF- α : protein – 10fold

mRNA – 58fold



G-CSF: protein – 50 fold

mRNA – 4000 fold



Levels of different protein concentration and mRNA level of selected cytokines

- μm - and nm-sized graphite after 72h incubation

Delta Ct (PCR cycles) vs. Housekeeping gene CDK2

			Csf2	Il10	Dnm1	Arhgap4	Ccl2	Arhgap26	Cc3	Tnf	Uchl1	Il12b	Csf3	Mrc1	Csf1	Cr2	Il1r1	Mst1r	Arhgap1	Il1b	Nos2
24h	NC		-4.95	-0.40	-3.51	-0.17	0.79	-1.66	-2.26	0.12	3.44	-0.78	3.02	-9.07	-0.85	5.25	4.96	7.77	-0.42	-1.16	-2.49
24h	Graphite	MX51- μm	-4.11	1.06	0.88	-0.22	3.11	-1.91	1.89	1.29	5.00	2.65	6.89	-5.76	2.34	6.02	2.12	3.02	3.02	-8.54	1.65
24h		P900- nm	-5.46	1.85	-0.12	-0.06	2.89	-0.36	1.85	1.05	4.35	1.41	5.79	-8.09	-0.52	8.93	3.91	2.55	0.75	-0.45	-0.56
72h	NC		-0.45	5.38	4.15	3.41	-1.49	8.80	0.91	-1.78	3.32	18.19	12.77	10.39	11.30	19.06	11.02	11.77	17.45	6.44	6.92
72h	Graphite	MX51 - μg	-2.38	-0.12	0.92	-0.93	-0.07	0.34	0.44	-0.34	0.35	-3.52	-2.95	3.68	1.60	1.56	0.59	-3.69	-3.62	0.68	0.19
72h		P900 - nm	2.40	-0.24	1.49	-0.59	1.14	0.80	2.28	0.44	0.00	0.62	-7.92	2.78	2.78	-3.78	1.59	2.89	-1.82	-0.38	-0.69

TNF- α

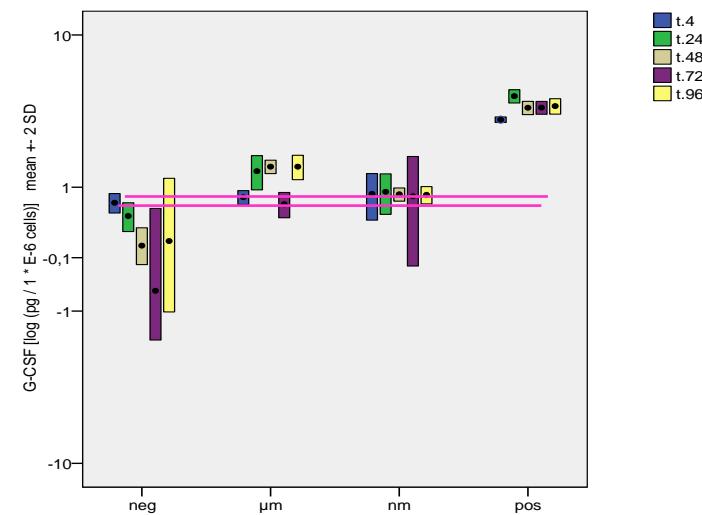
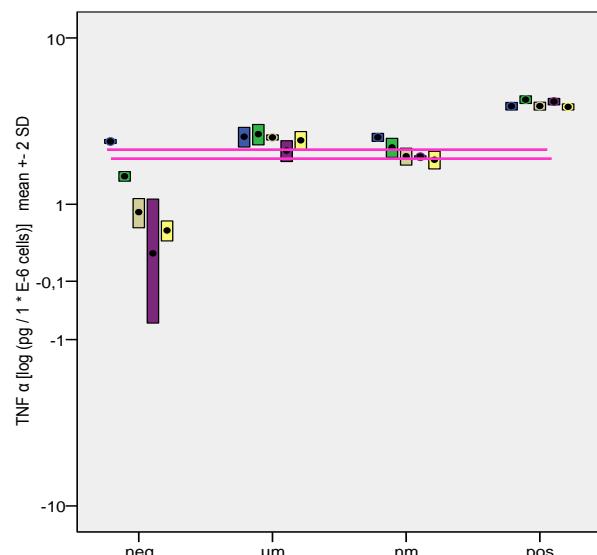
G-CSF

TNF- α : protein – 10fold

G-CSF: protein – 2fold

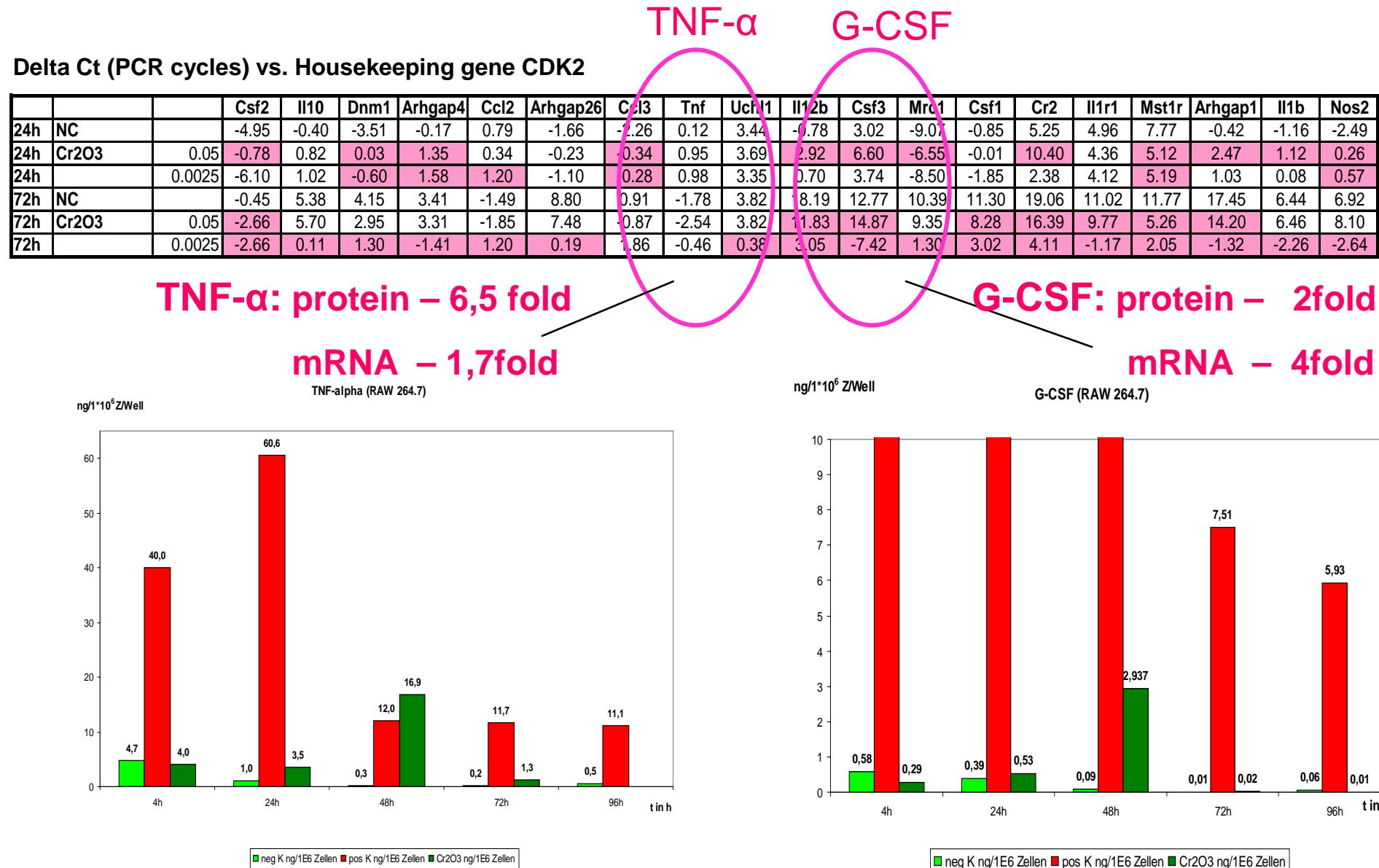
mRNA – 2fold

mRNA – 32fold



Levels of different protein concentration and mRNA level of selected cytokines

– different concentrations of nm-sized chromium (III) oxide after 72h incubation



Conclusions

Particles of different size distributions play a major role for failure of implant components.

In experiments with a macrophage cell line (RAW264.7) stimulated by model particles of corundum, graphite and Cr_2O_3 with nm and μm size and identical mass, different genes were up- or downregulated. Secreted cytokines and chemokines were effective to stimulate or inhibit cell growth, cell division or apoptosis and cell fusion. Nanometer size particles proved more active in secretion of proinflammatory cytokines than μm size particles.

An option to pharmacologically treat failing implants could be the inhibition of proinflammatory cytokines or chemokines.

Acknowledgements

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Freie Universität Berlin, CBF, Institut für Pathologie, M. Dilger-Rein
Institut für Statistik & Biometrie, W.Hopfenmüller

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2	Nos2	iNOS	Oxidative Stress	10
3	Arhgap1	Rho GTPase (ad)	IL-1 pathway	10
4	Csf3	G-CSF	Cytokine	10
5	Cr2	CD35 (CR2)	Complement	10
6	Dnm1	Dynamin 1	Complement	10
7	Mrc1	CD206	Phagocytose	9
8	Il12b	IL-12(p40)	Cytokine	9
9	Arhgap4	Rho GTPase (ad)	IL-1 pathway	8
10	Arhgap26	Rho GTPase (ad)	IL-1 pathway	7
11	Il10	IL-10	Cytokine	7
12	Il1r1	IL1-Receptor	IL-1 pathway	6
13	Uchl1	Ubiquitin	IL-1 pathway	6
14	Ccl3	MIP-1a	Cytokine	6
15	Il1b	IL-1b	Cytokine	6
16	Fcgr3a	CD16(FCgRIII)	Phagocytose	5
17	Msr1	SR-A (macrophage)	Phagocytose	5
18	Ctsh	Cathepsin H	IL-1 pathway	5
19	Tnfrsf1b	TNF Receptor 1	IL-1 pathway	5
20	Traf6	TRAF6	IL-1 pathway	5
21	Cd68	CD68	Fc-Receptors	5
22	Il15	IL15	Cytokine	5
23	Dnm2	Dynamin 2	Complement	5
24	Bax	Bax	Apoptose	5
25	Casp9	Caspase9	Apoptose	5

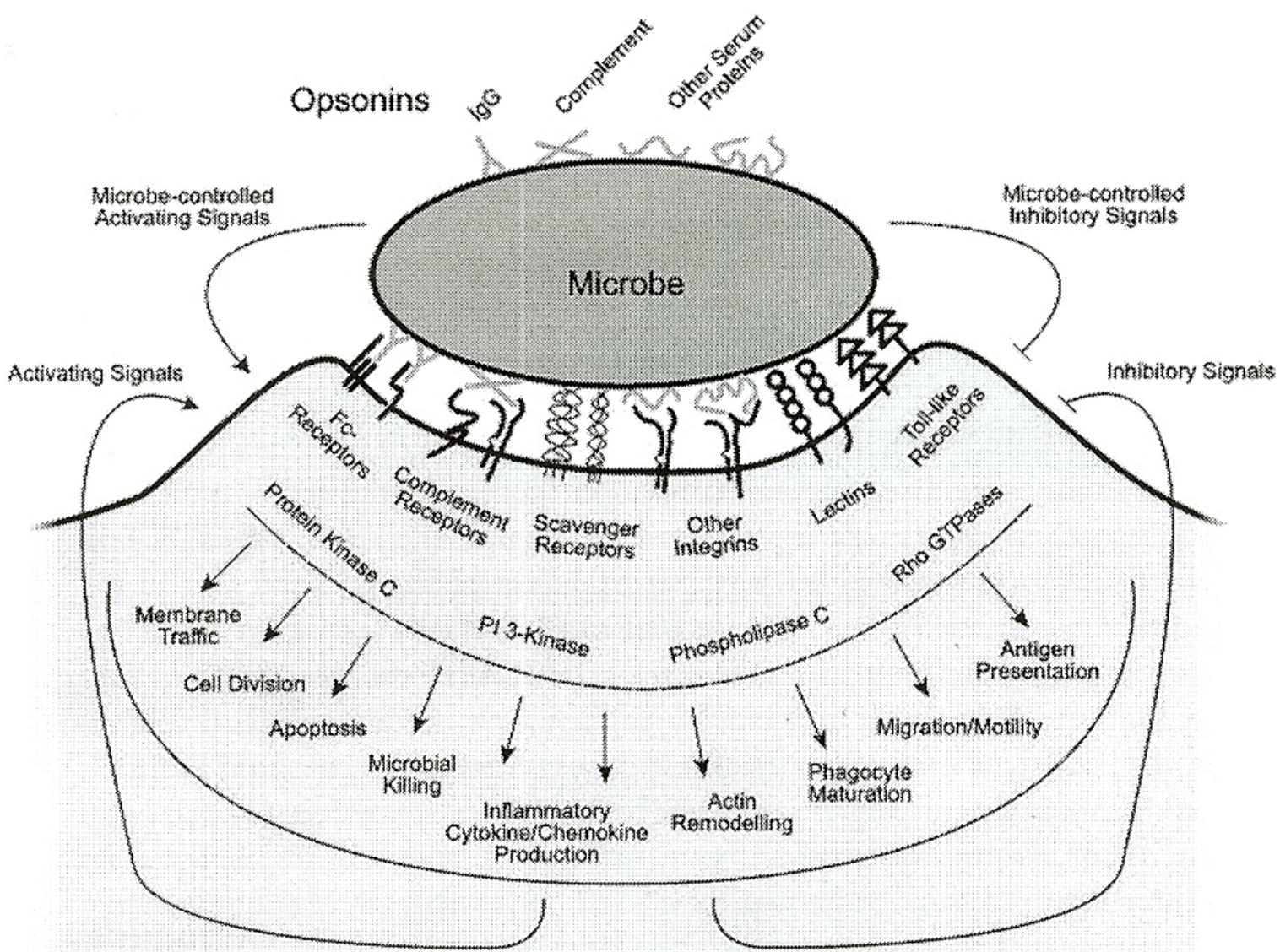
26	Cd14	CD14	Phagocytose	4
27	Hprt1	HPRT	Housekeeping genes	4
28	Ccl2	MCP-1	Cytokine	3
29	Csf2	GM-CSF	Cytokine	3
30	Fcgr1	CD64 (FCgRI)	Complement	3
31	Hip1	HIP1	Complement	3
32	Tlr4	TLR4	IL-1 pathway	2
33	Tnfrsf1a	TNF Receptor 1	IL-1 pathway	2
34	Il6	IL-6	Cytokine	2
35	Tnf	TNF alpha	Cytokine	2
36	Mst1r	CDw136	Phagocytose	1
37	Tnfrsf11a	TNF Receptor 1	IL-1 pathway	1
38	Fcgr2b	CD32 (FCgRII)	Fc-Receptors	1
39	Siglece	CD170	Complement	1
40	Ms4a2	(FCeRI)	Phagocytose	0
41	Irak1	IL-1-receptor ass	IL-1 pathway	0
42	Nfkb1	NF kappa B1	IL-1 pathway	0
43	Ptges2	Prostaglandin E	IL-1 pathway	0
44	Cdk2	CDK2 (cyclin-dependent kinase 2)	Housekeeping genes	0
45	Hc	C5a	Fc-Receptors	0
46	Bcl2	Bcl2	Apoptose	0
47	Casp3	Caspase3	Apoptose	0

			Receptor and signalling interactions	up-regulat 24h	down-regu 72h	up-regulat 72h	down-regu 72h	up-regulat Differenz	down-regu 24h-72h	unterschie Gesamt-
	Gene Symbol	Gene name								
32	Csf1	M-CSF	Cytokine	4	0	0	7	4	-7	11
45	Nos2	iNOS	Oxidative Stress	5	0	0	5	5	-5	10
41	Arhgap1	Rho GTPase (activating prot. 1)	IL-1 pathway	4	0	0	6	4	-6	10
26	Csf3	G-CSF	Cytokine	5	1	0	6	5	-5	10
33	Cr2	CD35 (CR2)	Complement	3	0	0	7	3	-7	10
6	Dnm1	Dynamin 1	Complement	6	0	0	4	6	-4	10
31	Mrc1	CD206	Phagocytose	4	0	0	5	4	-5	9
24	Il12b	IL-12(p40)	Cytokine	4	0	0	5	4	-5	9
7	Arhgap4	Rho GTPase (activating prot. 4)	IL-1 pathway	2	0	0	6	2	-6	8
12	Arhgap26	Rho GTPase (activating prot. 26)	IL-1 pathway	2	0	0	5	2	-5	7
11	18S	18S	Housekeeping genes	6	0	2	3	4	-3	7
4	Il10	IL-10	Cytokine	3	0	0	4	3	-4	7
39	Il1r1	IL1-Receptor	IL-1 pathway	2	3	0	7	2	-4	6
23	Uchl1	Ubiquitin	IL-1 pathway	1	0	0	5	1	-5	6
20	Ccl3	MIP-1a	Cytokine	7	0	1	0	6	0	6
42	Il1b	IL-1b	Cytokine	2	1	0	5	2	-4	6
29	Fcgr3a	CD16(FCgRIII)	Phagocytose	1	0	0	4	1	-4	5
35	Msrl	SR-A (macrophage scavenger r l)	Phagocytose	0	0	5	0	-5	0	5
38	Ctsh	Cathepsin H	IL-1 pathway	0	1	0	6	0	-5	5
34	Tnfrsf1b	TNF Receptor 1B (Receptor 2)	IL-1 pathway	6	0	1	0	5	0	5
36	Traf6	TRAF6	IL-1 pathway	0	0	0	5	0	-5	5
28	Cd68	CD68	Fc-Receptors	0	0	5	0	-5	0	5
47	Il15	IL15	Cytokine	1	0	0	4	1	-4	5
17	Dnm2	Dynamin 2	Complement	5	0	0	0	5	0	5
37	Bax	Bax	Apoptose	0	0	5	0	-5	0	5
21	Casp9	Caspase9	Apoptose	0	0	0	5	0	-5	5

Nr.	Gene Symbol	Gene name	Receptor and signalling interactions	Differentially regulated genes
1	Csf1	M-CSF	Cytokine	11
2	Nos2	iNOS	Oxidative Stress	10
3	Arhgap1	Rho GTPase (ad)	IL-1 pathway	10
4	Csf3	G-CSF	Cytokine	10
5	Cr2	CD35 (CR2)	Complement	10
6	Dnm1	Dynamin 1	Complement	10
7	Mrc1	CD206	Phagocytose	9
8	Il12b	IL-12(p40)	Cytokine	9
9	Arhgap4	Rho GTPase (ad)	IL-1 pathway	8
10	Arhgap26	Rho GTPase (ad)	IL-1 pathway	7
11	18S	18S	Housekeeping genes	7
12	Il10	IL-10	Cytokine	7
13	Il1r1	IL1-Receptor	IL-1 pathway	6
14	Uchl1	Ubiquitin	IL-1 pathway	6
15	Ccl3	MIP-1a	Cytokine	6
16	Il1b	Il-1b	Cytokine	6
17	Fcgr3a	CD16(FCgRIII)	Phagocytose	5
18	Msr1	SR-A (macrophage)	Phagocytose	5
19	Ctsh	Cathepsin H	IL-1 pathway	5
20	Tnfrsf1b	TNF Receptor 1B	IL-1 pathway	5
21	Traf6	TRAFF6	IL-1 pathway	5
22	Cd68	CD68	Fc-Receptors	5
23	Il15	IL15	Cytokine	5
24	Dnm2	Dynamin 2	Complement	5
25	Bax	Bax	Apoptose	5
26	Casp9	Caspase9	Apoptose	5
27	Cd14	CD14	Phagocytose	4
28	Hprt1	HPRT	Housekeeping genes	4
29	Ccl2	MCP-1	Cytokine	3
30	Csf2	GM-CSF	Cytokine	3
31	Fcgr1	CD64 (FCgRI)	Complement	3
32	Hip1	HIP1	Complement	3

Nr.	Function	Genome	Genename
1	Housekeeping genes	CDK2 (cyclin-dependent kinase 2)	CDK2
2	Housekeeping genes	GAPDH	Gapdh
3	Housekeeping genes	HPRT	HPRT1
4	Apoptose	Bax	BAX
5	Apoptose	Bcl2	BCL2
6	Apoptose	Caspase3	CASP3
7	Apoptose	Caspase9	CASP9
8	Oxidativer Stress	iNOS	iNOS
9	Phagocytose	CDw136	MST1R
10	Phagocytose	CD206	MRC1
11	Phagocytose	(FCeRI)	MS4A2
12	Phagocytose	CD14	CD14
13	Phagocytose	CD16(FCgRIII)	FCGR3A
14	<i>Fc-Rezeptoren</i>	C5a	HC
15	<i>Fc-Rezeptoren</i>	CD68	Cd68
16	<i>Fc-Rezeptoren</i>	CD32 (FCgRII)	FCGR2B
17	<i>Complement</i>	CD35 (CR2)	Cr2
18	<i>Complement</i>	CD64 (FCgRI)	Fcgr1
19	<i>Complement</i>	CD170	Siglece
20	<i>Complement</i>	SR-A (macrophage scavenger r I)	MSR1
21	<i>Complement</i>	HIP1	HIP1
22	<i>Complement</i>	Dynamin 1	DNM1
23	<i>Complement</i>	Dynamin 2	DNM2

24	Cytokine	G-CSF	CSF3
25	Cytokine	GM-CSF	CSF2
26	Cytokine	IL-10	IL10
27	Cytokine	Il-1b	IL1B
28	Cytokine	Il-6	IL6
29	Cytokine	IL-12(p40)	IL12p40
30	Cytokine	MCP-1	CCL2
31	Cytokine	Prostaglandin E Synthasse 2	Ptges2
32	Cytokine	TNF alpha	TNF
33	Cytokine	MIP-1a	Ccl3
34	Cytokine	IL15	IL15
35	Cytokine	M-CSF	Csf1
36	IL-1 pathway	IL1-Rezeptor	IL1R1
37	IL-1 pathway	IL1-receptor associated kinase1	IRAK1
38	IL-1 pathway	TNF Receptor 1A (receptor 1)	TNFRSF1A
39	IL-1 pathway	TNF Receptor 1B (Receptor 2)	TNFRSF1B
40	IL-1 pathway	TRAF6	TRAF6
41	IL-1 pathway	Ubiquitin	UCHL1
42	IL-1 pathway	Rho GTPase (activating prot. 1)	ARHGAP1
43	IL-1 pathway	Rho GTPase (activating prot. 4)	ARHGAP4
44	IL-1 pathway	Rho GTPase (activating prot. 26)	ARHGAP26
45	IL-1 pathway	Rho GTPase (activating prot. 29)	ARHGAP29
46	IL-1 pathway	Cathepsin H	CTSH
47	IL-1 pathway	TLR4	Tlr4
48	IL-1 pathway	NF kappa B1	NFKB1



modified from D.M.Underhill and A.Ozinsky, Annu.Rev.Immunol.2002.20:825-852

Mikrokorundum:

MR 52 (Al_2O_3):

Hersteller und Lieferant: Martinswerk

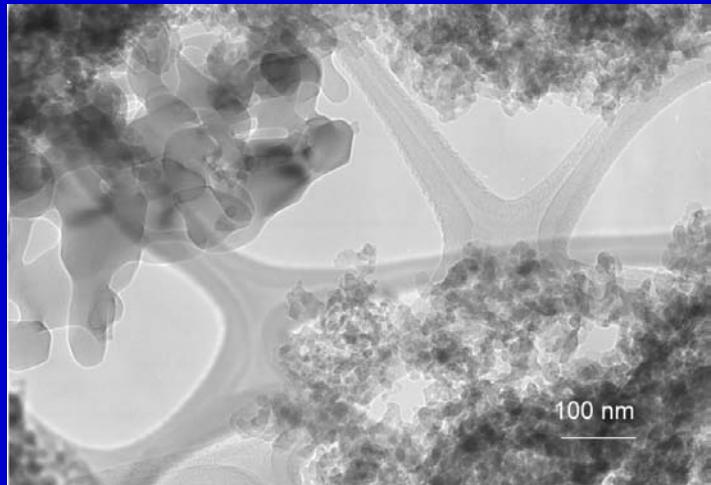
GmbH

Manufacturer: particles made according to Bayer-Verfahren (alkalischer Aufschluss von Bauxit, Gewinnung von $\text{Al}(\text{OH})_3$, Kalzinierung). Primary size 1,5 μm (99,8 % Al_2O_3 ; $\leq 0,1$ % Na_2O ; $\leq 0,05$ % CaO ; $\leq 0,04$ % Fe_2O_3 ; $\leq 0,07$ % SiO_2)

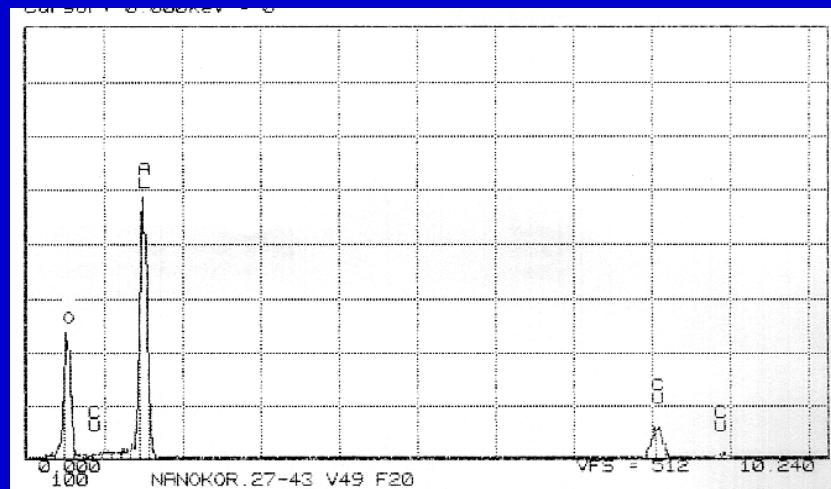
SEM MR52
I. Dörfel (BAM)

M. Gemeinert (BAM): diameter of primary particles around 1.5 μm (SEM), specific surface 5,94 m^2/g (BET-technique), density 3.94 g/cm^3 .

Nanocorundum LA (Al_2O_3): Auer-Remy Vertriebsgesellschaft
Seltene Erden, H. Blum & Co. GmbH; Manufacturer: Nanostructured &
Amorphous Materials Inc. (USA). Primary size 27 - 43 nm, specific surface
35 m²/g, density 3,97 g/cm³. (99,5% α - Al_2O_3 ; 0,3 % Fe_2O_3 ; 0,001 % MgO;
0,05 % SiO_2)

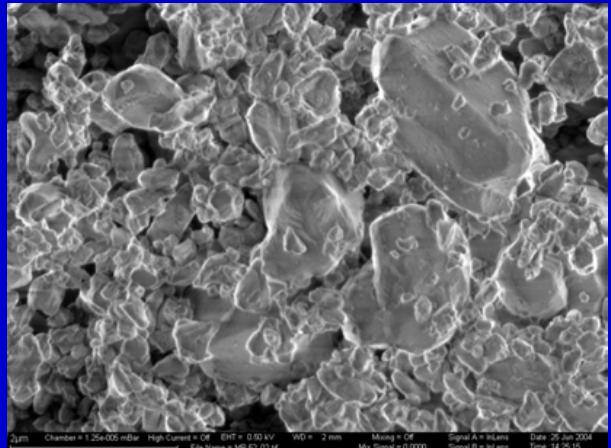


TEM; Nanocorundum (LA)
I. Dörfel (BAM)

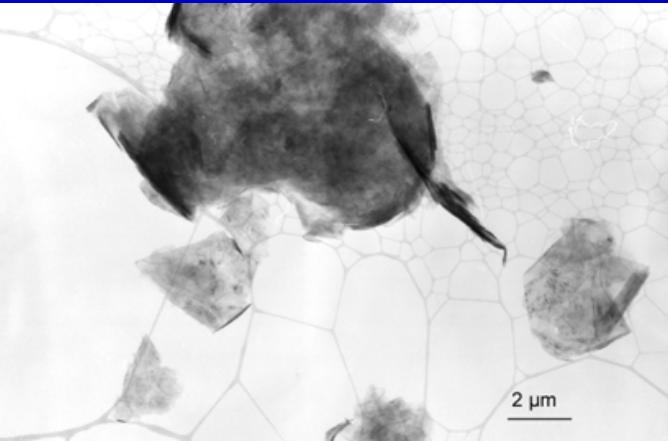


EDX-Spektrum; Nanocorund (LA)
I. Dörfel (BAM)

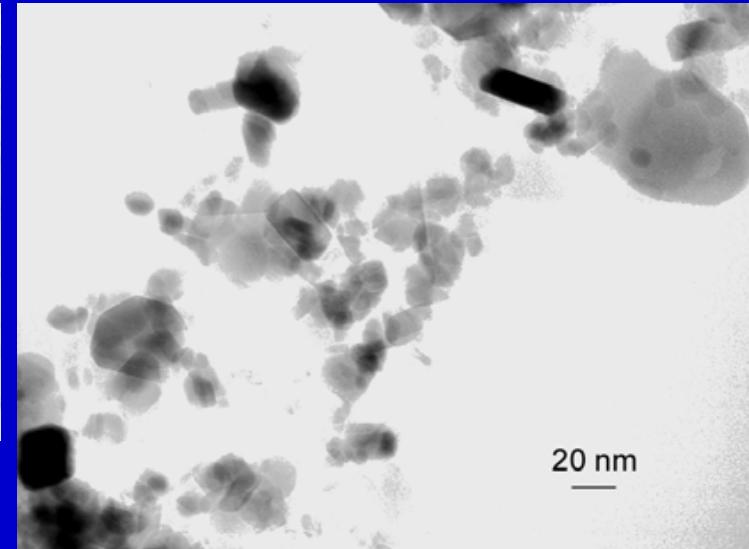
M. Gemeinert (BAM): diameter of primary particles < 100 nm (TEM)
specific surface 36 m²/g (BET-technique), density 3.677 g/cm³
EDX-spectrum aluminia (copper from the nets)



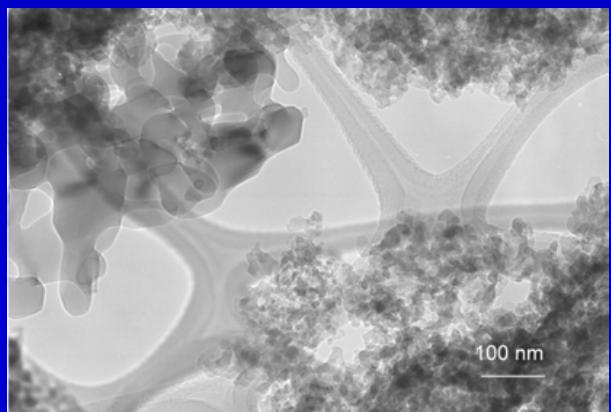
micro corundum sample MR 52



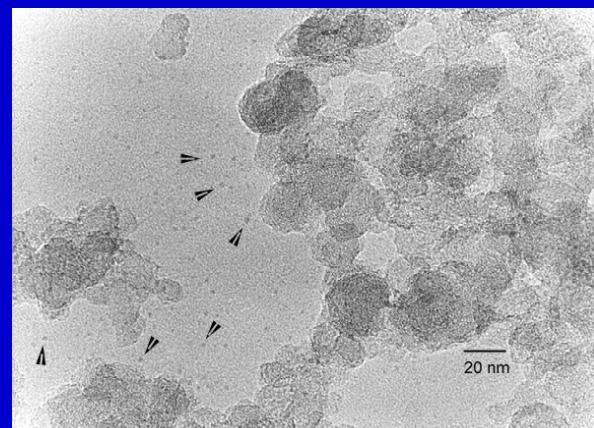
micro graphite sample MX 51



nano-chromium-III-oxide



nanocorundum sample NAM



nanographite Printex 90C