



Influence of Centrifugation on Morphology of UHMWPE Wear Particles

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Motivation:

The use of ultracentrifugation or centrifugation to separate plastic debris from digestion residues may lead to the alteration of particle morphology and loss of a substantial fraction of particles as we observed...

M. Visentin et al: A new method for isolation of polyethylene wear debris from tissue and synovial fluid. *Biomaterials* **25** (2004) 5531-5537.



Our isolation method

3 g of freeze-dried tissue

acid hydrolysis HNO_3

washing – 2x with HNO_3

2x with H_2O

centrifugation

500 x g

1 min

wear particles were diluted with isopropyl alcohol

pre-filtration through 10 μm polytetrafluorethylene (PTFE) membrane




filtration through 0.1 polycarbonate (PC) membrane



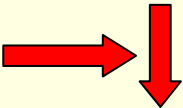


SEM



What could happen during centrifugation?

Model PE particle Image analysis parameters			
Equivalent diameter	1.13	1.13	0.63
Circularity	0.09	0.12	0.26
Elongation	16.74	1.49	6.39

Change of image analysis parameters

$\langle D \rangle$	
$\langle C \rangle$	
$\langle E \rangle$	

Summary of studied implants

Case	Sex	Birth	Implant type	Implant duration
H1	M	1940	ABG (Howmedica)	8.5 yrs
H2	F	1955	ABG (Howmedica)	9 yrs
H3	F	1933	Poldi/Ultima	8 yrs
K1	M	1927	PFC (J+J)	8 yrs

All patients: elongated particles & very similar size distributions.



Modified isolation method

3 g of freeze-dried tissue

acid hydrolysis HNO_3

washing – 2x with HNO_3

2x with H_2O

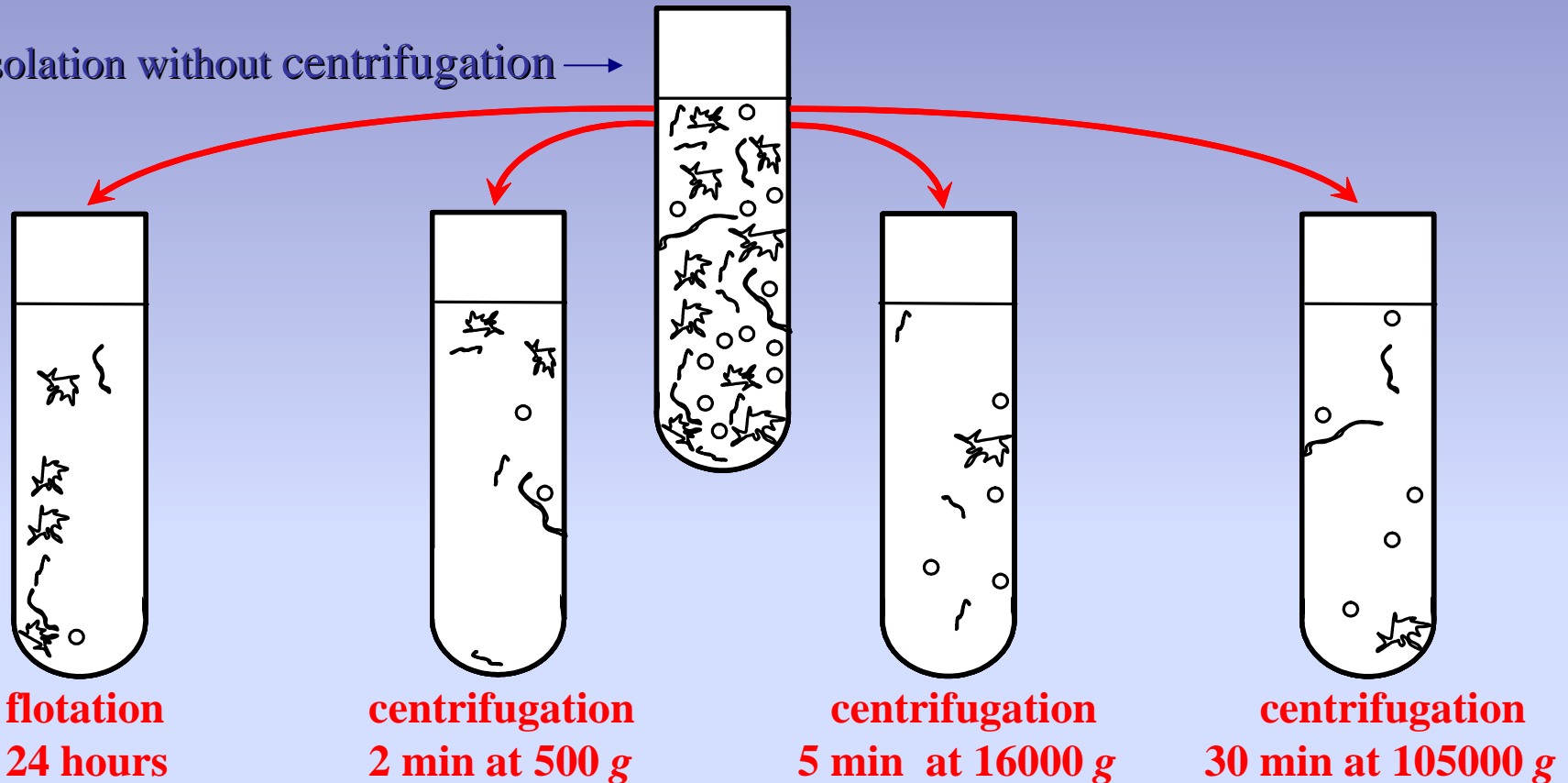
flotation

24 hours



Adjustment of the experiment

isolation without centrifugation →



wear particles were diluted with isopropyl alcohol

pre-filtration through 10 μm PTFE membrane

filtration through 0.1 μm PC membrane



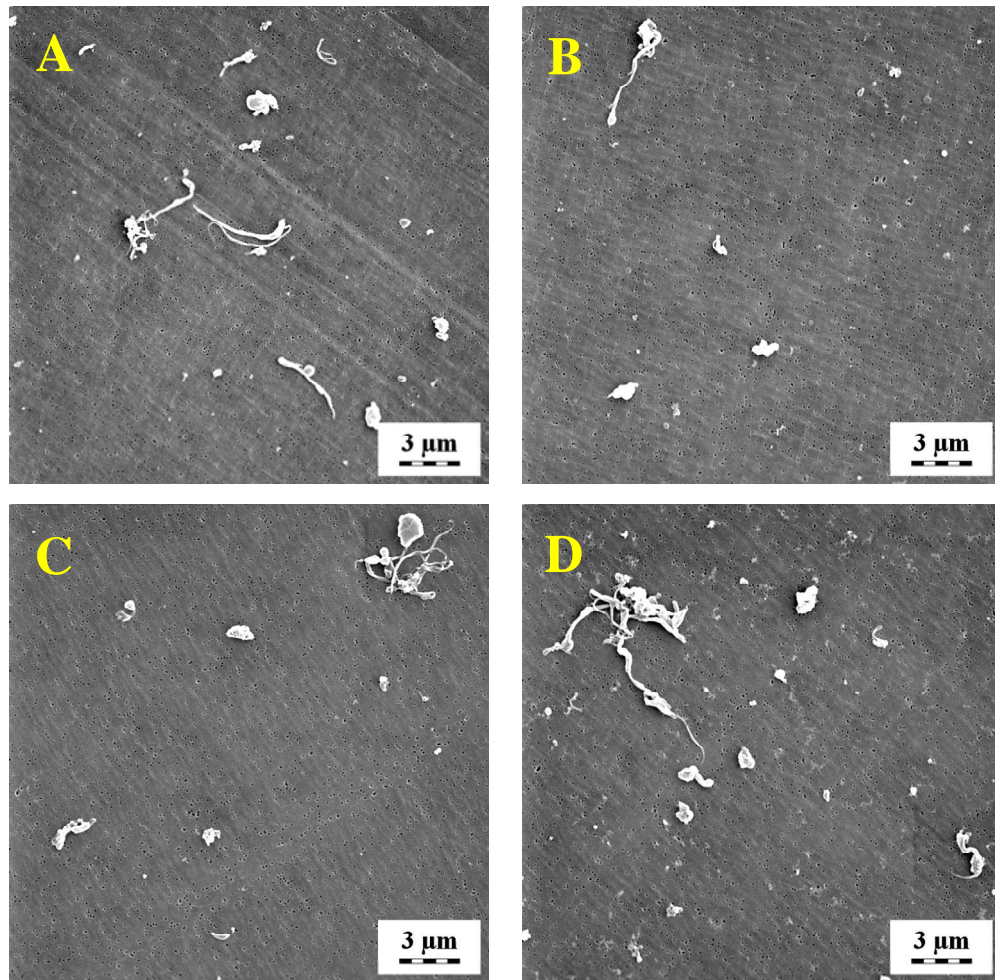
SEM



SEM micrographs: elongated particles

patient H1

wear particles (0.1 – 10 μm)



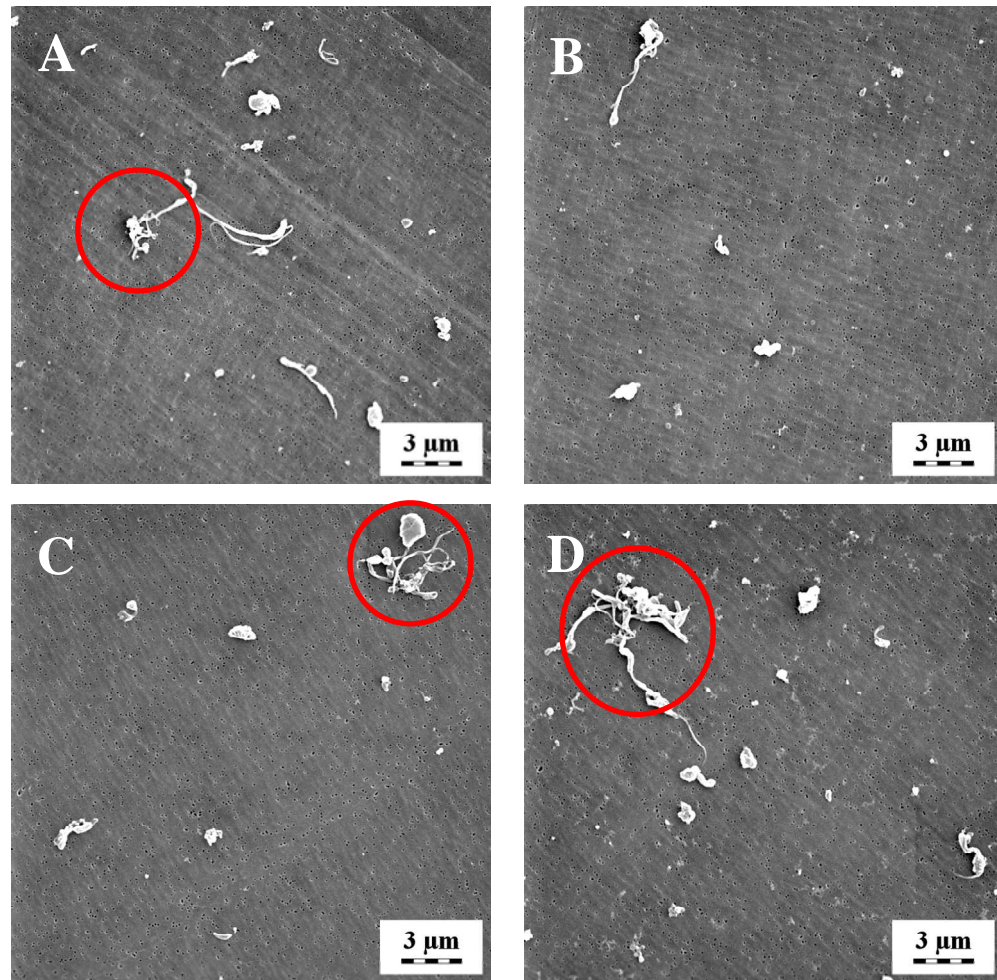
A) flotation

B) centrifugation 2 min at 500 g

C) centrifugation 5 min at 16 000 g

D) centrifugation 30 min at 105 000 g

Typical SEM micrographs



selected micrographs of
wear particles (0.1 – 10 μm)

A) flotation

B) centrifugation 2 min at 500 g

C) centrifugation 5 min at 16 000 g

D) centrifugation 30 min at 105 000 g

Each sample = **20** micrographs

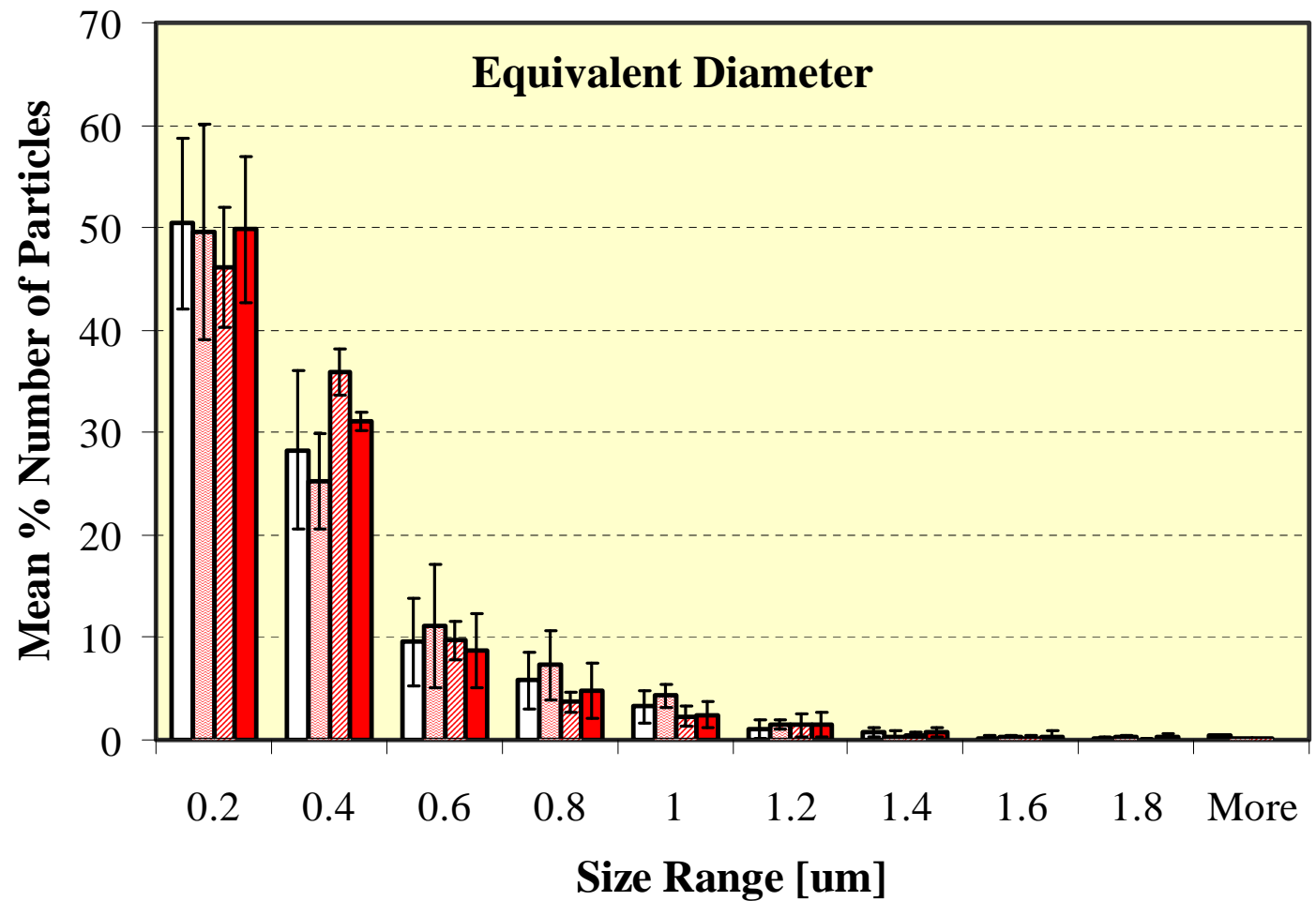
540 μm² = area of 1 micrograph

420 = average number of particles
for each method of isolation

agglomerates were excluded



Results



flotation



centrifugation: 2 min at 500 g



centrifugation: 5 min at 16000 g

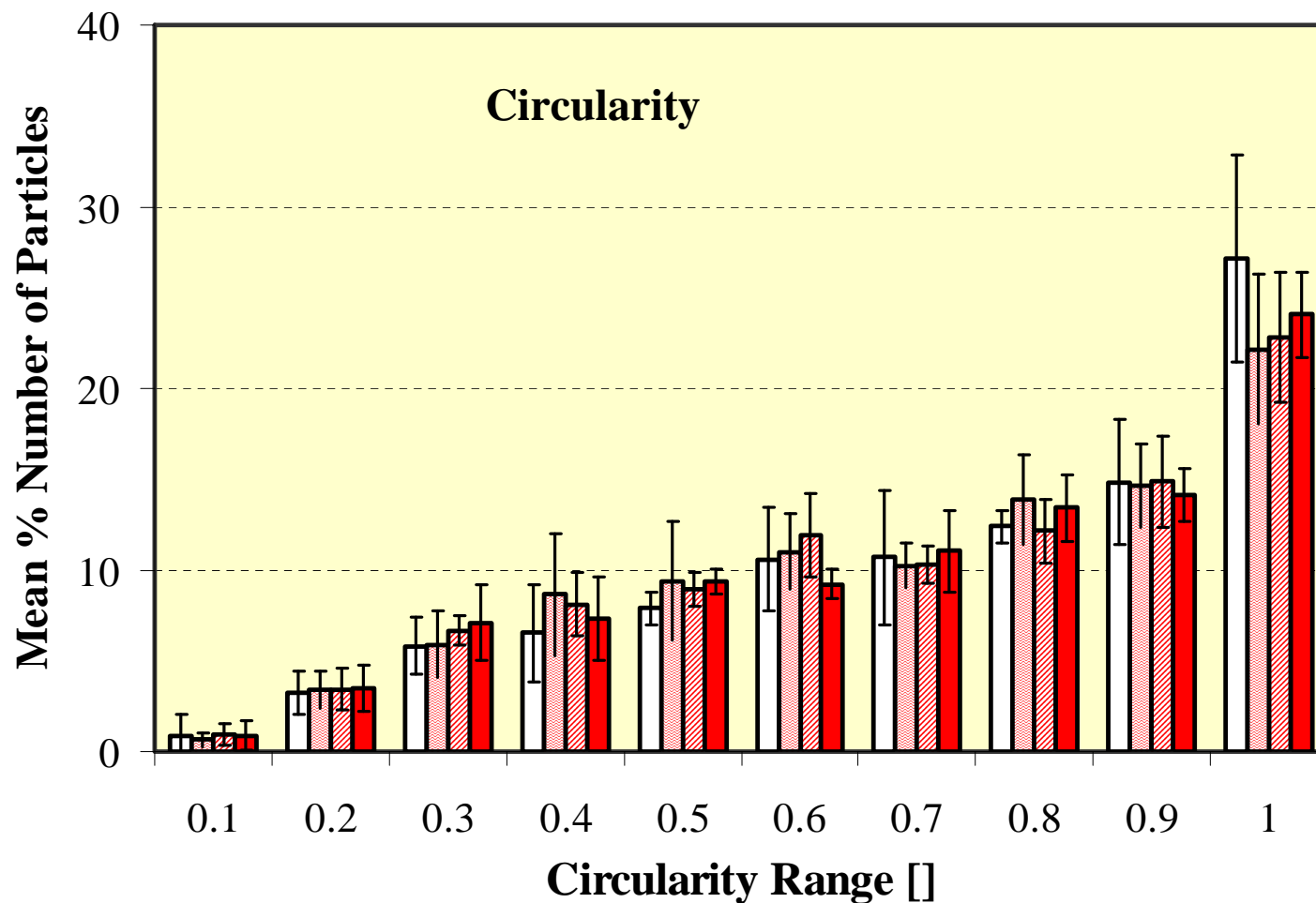


centrifugation: 30 min at 105000 g

* each column represents an average through all four patients



Results



flotation



centrifugation: 2 min at 500 g



centrifugation: 5 min at 16000 g

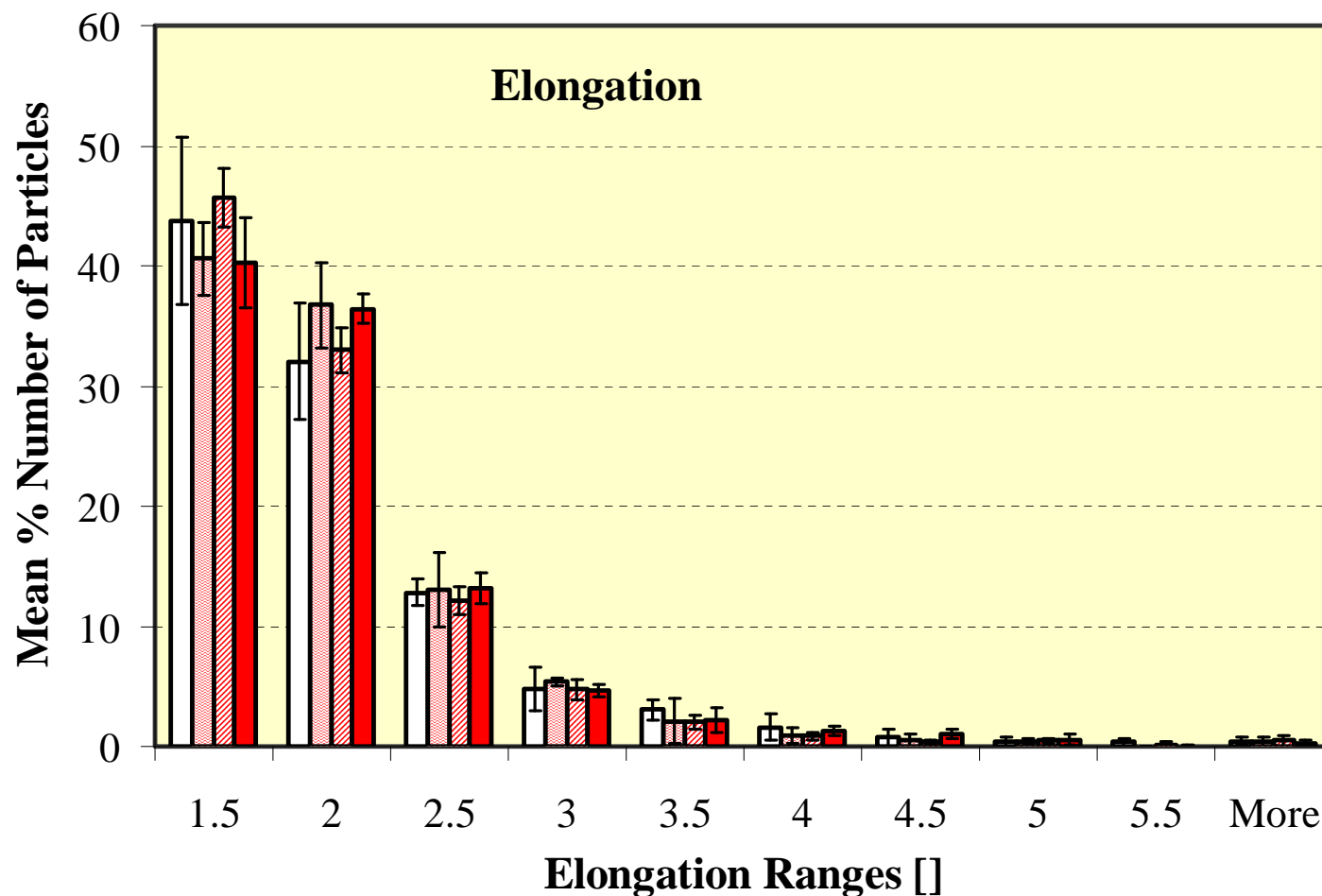


centrifugation: 30 min at 105000 g

* each column represents an average through all four patients



Results



flotation



centrifugation: 2 min at 500 g



centrifugation: 5 min at 16000 g



centrifugation: 30 min at 105000 g

* each column represents an average through all four patients



The 1st Conclusion:

The centrifugation does not affect morphology of isolated *in vivo* wear particles of UHMWPE.



New experiment with different centrifugation

3 g of freeze dried tissue

acid hydrolysis HNO_3

washing – 2x with HNO_3

2x with H_2O

wear particles were diluted with isopropyl alcohol

pre-filtration through 10 μm PTFE membrane

filtration through 0.1 μm PC membrane



centrifugation

500 x g

1 min

centrifugation

2000 x g

5 min

SEM

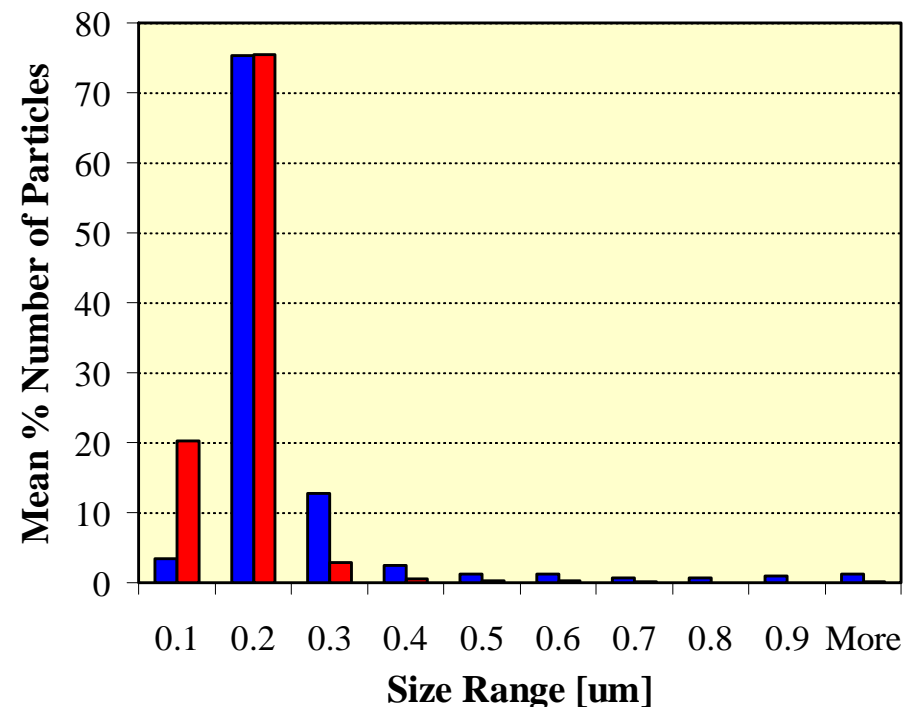
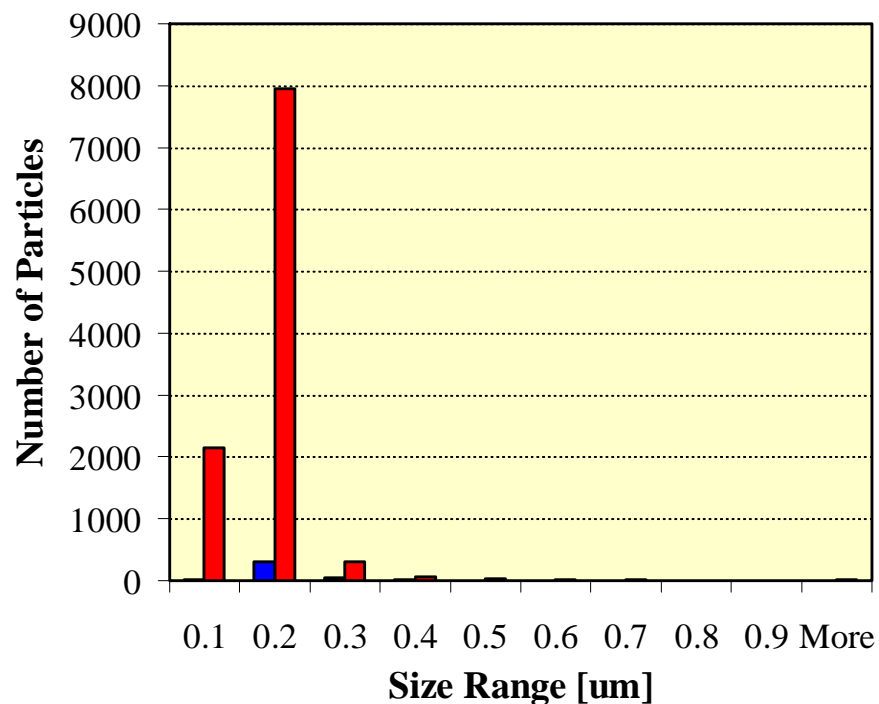


Results for the 1st sample

 centrifugation: 1 min at 500 g; 490 particles; $\langle D \rangle = 0.14 \mu\text{m}$

 centrifugation: 5 min at 2000 g; 10540 particles; $\langle D \rangle = 0.12 \mu\text{m}$

Equivalent Diameter

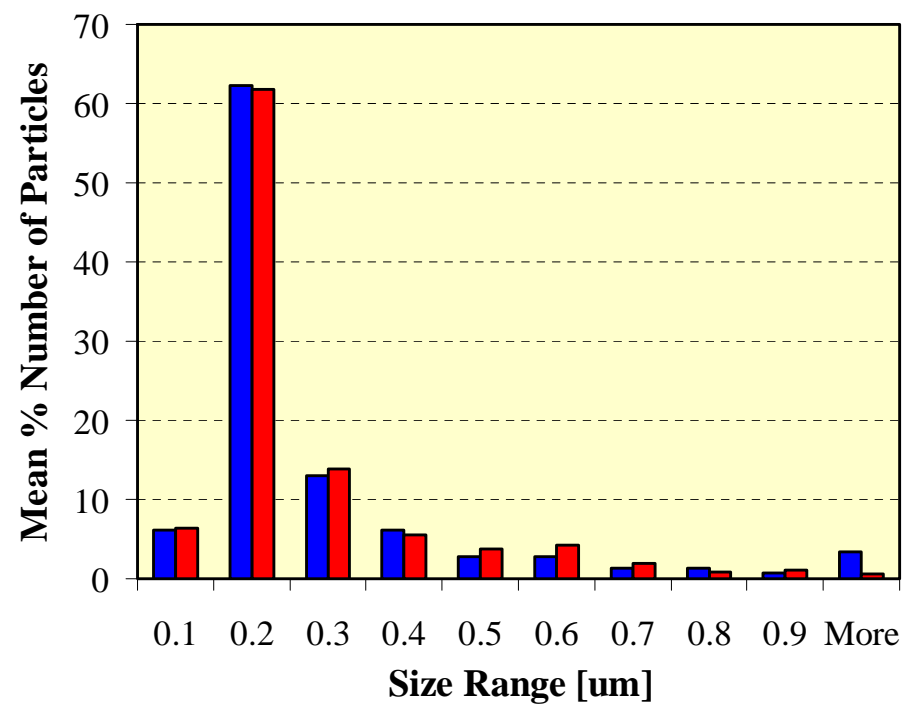
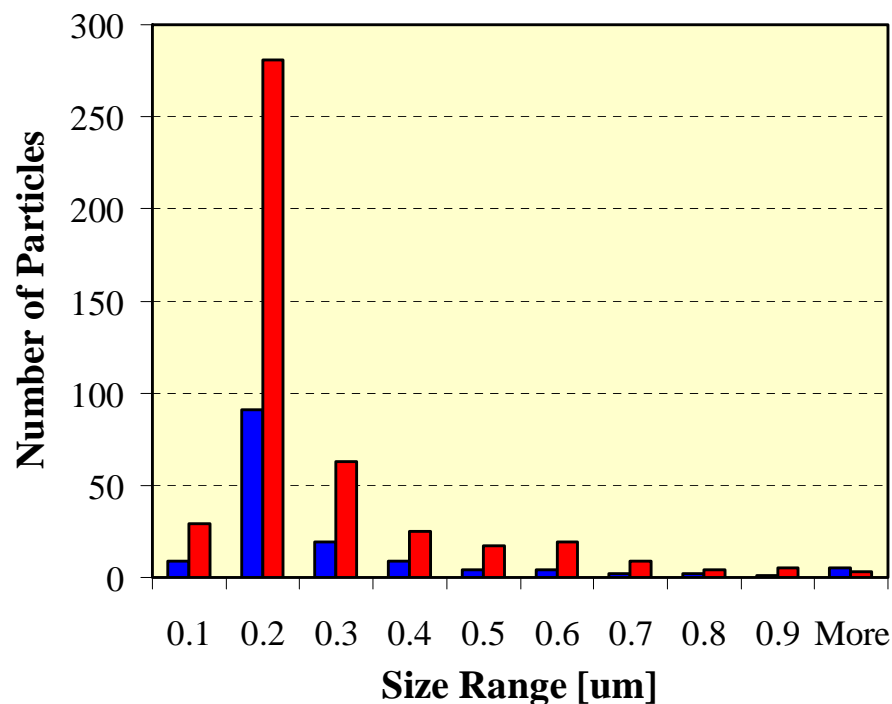


Results for the 2nd sample

 centrifugation: 1 min at 500 g; 146 particles; $\langle D \rangle = 0.24 \mu\text{m}$

 centrifugation: 5 min at 2000 g; 455 particles; $\langle D \rangle = 0.22 \mu\text{m}$

Equivalent Diameter



The 2nd Conclusion:

1. Centrifugation does not affect morphology of the isolated *in vivo* wear particles of UHMWPE due to collisions...

BUT

2. ...in special cases, a higher centrifugation speed results in a higher yield of small particles. This may influence the final distributions determined by image analysis.



Thank you for your attention.

