Influence of Centrifugation on Morphology of UHMWPE Wear Particles

Monika Lapčíková\(^{(a)}\)

J. Hromádková\(^{(a)}\), M. Šlouf\(^{(a)}\), Z. Fejfarková\(^{(b)}\),
E. Zolotarevová\(^{(b)}\), G. Entlicher\(^{(b)}\), D. Pokorný\(^{(c)}\), A. Sosna\(^{(c)}\)

\(^{(a)}\) Institute of Macromolecular Chemistry AS CR v.v.i.
\(^{(b)}\) Faculty of Sciences, Charles University
\(^{(c)}\) Orthopedics Clinic, Faculty Hospital Motol
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...

Our isolation method

3 g of freeze-dried tissue

acid hydrolysis HNO$_3$

washing – 2x with HNO$_3$

2x with H$_2$O

wear particles were diluted with isopropyl alcohol

pre-filtration through 10 µm polytetrafluoroethylene (PTFE) membrane

filtration through 0.1 polycarbonate (PC) membrane

centrifugation

500 x g

1 min

SEM

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What could happen during centrifugation?

<table>
<thead>
<tr>
<th>Image analysis parameters</th>
<th>Model PE particle</th>
<th>Change of image analysis parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equivalent diameter</td>
<td>1.13</td>
<td>〈D〉</td>
</tr>
<tr>
<td>Circularity</td>
<td>0.09</td>
<td>〈C〉</td>
</tr>
<tr>
<td>Elongation</td>
<td>16.74</td>
<td>〈E〉</td>
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Summary of studied implants

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Birth</th>
<th>Implant type</th>
<th>Implant duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>M</td>
<td>1940</td>
<td>ABG (Howmedica)</td>
<td>8.5 yrs</td>
</tr>
<tr>
<td>H2</td>
<td>F</td>
<td>1955</td>
<td>ABG (Howmedica)</td>
<td>9 yrs</td>
</tr>
<tr>
<td>H3</td>
<td>F</td>
<td>1933</td>
<td>Poldi/Ultima</td>
<td>8 yrs</td>
</tr>
<tr>
<td>K1</td>
<td>M</td>
<td>1927</td>
<td>PFC (J+J)</td>
<td>8 yrs</td>
</tr>
</tbody>
</table>

All patients: elongated particles & very similar size distributions.
Modified isolation method

3 g of freeze-dried tissue

acid hydrolysis HNO₃

washing – 2x with HNO₃

2x with H₂O

flotation

24 hours
Adjustment of the experiment

Isolation without centrifugation

- Flotation for 24 hours
- Centrifugation for 2 min at 500 g
- Centrifugation for 5 min at 16000 g
- Centrifugation for 30 min at 105000 g

Wear particles were diluted with isopropyl alcohol.

Pre-filtration through 10 µm PTFE membrane.

Filtration through 0.1 PC membrane.

SEM

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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

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

selected micrographs of wear particles (0.1 – 10 µm)

Each sample = 20 micrographs

540 µm² = area of 1 micrograph
420 = average number of particles
for each method of isolation
agglomerates were excluded
Results

Equivalent Diameter

Mean % Number of Particles

Size Range [um]

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

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Results

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- Flotation
- Centrifugation: 2 min at 500 g
- Centrifugation: 5 min at 16000 g
- Centrifugation: 30 min at 105000 g

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Results

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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

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The 1\textsuperscript{st} Conclusion:

The centrifugation does not affect morphology of isolated \textit{in vivo} wear particles of UHMWPE.
New experiment with different centrifugation

3 g of freeze dried tissue

acid hydrolysis HNO₃

washing – 2x with HNO₃

2x with H₂O

wear particles were diluted with isopropyl alcohol

pre-filtration through 10 µm PTFE membrane

filtration though 0.1 PC membrane

SEM

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Results for the 1st sample

- Centrifugation: 1 min at 500 g; 490 particles; $<D> = 0.14 \, \mu m$
- Centrifugation: 5 min at 2000 g; 10540 particles; $<D> = 0.12 \, \mu m$

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Results for the 2\textsuperscript{nd} sample

Centrifugation: 1 min at 500 g; 146 particles; \(\langle D \rangle = 0.24 \mu m\)

Centrifugation: 5 min at 2000 g; 455 particles; \(\langle D \rangle = 0.22 \mu m\)

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The 2nd Conclusion:

1. Centrifugation does not affect morphology of the isolated \emph{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.