Functional Nanoparticles for Selective Recognition of Biomarkers of Oxidative Stress

Michael Lämmerhofer
1Institute of Pharmaceutical Sciences, University of Tübingen, Germany;

Kontakt:
Professor für Pharmazeutische Analytik und Bioanalytik
Pharmazeutisches Institut, Eberhard Karls Universität Tübingen
Auf der Morgenstelle 6, 72076 Tübingen
T +49 7071 29 76793, F +49 7071 29 4565
E-mail: michael.laemmerhofer@uni-tuebingen.de

Sources and Cellular Responses to Reactive Oxygen Species (ROS)

Endogenous sources
Mitochondria Peroxidases Lipoperoxides NADPH oxidases Cycloxygenase (PGH)

Exogenous sources
Ultraviolet light Ionizing radiation Chemotherapeutics Environmental toxins

ROS
defences
"NO, O2 •, "OH

less
Increased physiological function
Decreased cellular proliferation Defective host defences
Decreased cell death
Normal growth and metabolism
Decreased physiological function

more

Hemicellulosis

Molecular Complexity of Oxidized Phospholipids (oxPLs)

PC, Phosphocholine

4-Hydroxynonenal

Malondialdehyde

Source: Toren Finkel and Niles J. Hollstein, Nature 46, 238-245 (9 November 2000) doi:10.1038/35041687

Buchkow et al. ANTIOXIDANTS & REDOX SIGNALING 2016, 12, 1009-1003
Role of Oxidized Phospholipids and Oxidative Stress

- Atherosclerosis
- Diabetes
- Multiple Sclerosis (MS)
- Systemic Lupus Erythematosus
- Rheumatoid Arthritis
- Alzheimer Disease
- Parkinson’s Disease

Oxidized LDL and its Role in Atherosclerosis

doi:10.1038/nm0603-641 Autoimmunity: busting the atherosclerotic plaque
Noel Rosed, Marina Afanasyeva

Extraction of Oxidized LDL as Biomarkers

- Random Immobilization
- Oriented Immobilization
- Adsorptive Binding
- Covalent linkage via amide coupling (PEG7 Spacer)
- Via carbohydrate residue and hydrazide coupling

Antibody Immobilization Strategies
Characterization by DLS, ζ-Potentials and UV

Specificity of GNP-cProtA-Ab

Clinical Analysis of Biomarkers of Oxidative Stress by MALDI-TOF

Chemical Trapping Concept
Application to MDA-LDL and CuLDL

<table>
<thead>
<tr>
<th>PONPC in MDA-LDL</th>
<th>PONPC in MDA-LDL</th>
<th>PONPC in CuLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean [ng/µg]</td>
<td>1.24 ± 0.26</td>
<td>0.50 ± 0.10</td>
</tr>
</tbody>
</table>

OxPL OxPL OxPL OxPL

1. MeOH extraction
2. PAs derivatization
3. Trapping (GNPs)
4. NH$_2$-OH
5. LC-MS

Outlook
- Development of method for comprehensive analysis of oxidized phospholipids
- Resolution of full structural complexity (incl isomers)
- Method for distinction between enzymatic oxidation and random oxidative modification

Collaborations
- LC-QTOF-MS/MS Analysis (e.g. mult-target inflammation marker analysis, for validation purposes)
- Functionalized/engineered nanoparticles for molecular recognition and imaging purposes

Functionalyzed Engineered Nanoparticles for Imaging

<table>
<thead>
<tr>
<th>Type of Material</th>
<th>Fe$_2$O$_3$</th>
<th>Gold</th>
<th>Silver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control of Size</td>
<td>sodium citrate / HAuCl$_4$ (C/H) ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imaging Modality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT Gold, Silver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>Iron-Oxide, Gd$^{3+}$/Silica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optical</td>
<td>Quantum dots, SERS-active NPs (Gold, Silver)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multifunctionality (Drug-load)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chemical Surface Functionalization
- Molecular Recognition
- Passivation
- Biccompatibility
- Fluorescence Tag
- Kinetic Properties