Prions – A Challenge and Informative Paradigm for the Cleaning and Disinfection of Medical Devices

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Prion Diseases: Transmissible Spongiform Encephalopathies of Animals and Humans

- **Scrapie**
  - Sheep and goat
- **Bovine Spongiform Encephalopathy (BSE)**
  - Cattle
- **Chronic Wasting Disease (CWD)**
  - Deer and elk
- **Creutzfeldt-Jakob Disease (CJD)**
  - Human
- **Variant Creutzfeldt-Jakob Disease (vCJD)**
  - Human
Prion Disease Characteristics in the Central Nervous System

- Replication of a transmissible agent (infectivity)
- Accumulation of prion protein (PrP) with an aberrant conformation
- Neurodegeneration

PrPC

PrPSc/PrPTSE

- Misfolded
- Aggregated
- Protease-resistant
- Associated with infectivity
Detection of Normal and Pathological Prion Protein by Western Blotting

- PrPC
  - PK
  - PK

- PrP^TSE
  - PK
  + PK

PrP sen

PrP res

Western blots of PrP from hamsters (mAb 3F4)

35 kDa
30 kDa
PrP 27-30 (MW of diglycosylated band)
Etiology of Prion Diseases

- According to the prion hypothesis, transmissible spongiform encephalopathies are caused by a novel biological principle of infection – "Prions".

- Prions are "proteinaceous infectious particles" consisting essentially of misfolded, aggregated prion protein (PrP\textsuperscript{TSE}) which is derived from a host-encoded cellular precursor (PrP\textsuperscript{C}).

Govaerts et al., 2004, Proc Natl Acad Sci USA, 101: 8342-8347
Prion Replication by Seeded Polymerization of the Prion Protein

Brundin et al., 2010, Nat Rev Mol Cell Biol, 11: 301-307
Watts et al., 2006, PLoS Pathog, 2: e26
Tolerance of Prions to Disinfection Procedures

<table>
<thead>
<tr>
<th>No detectable infectivity</th>
<th>Significant titre reduction</th>
<th>Little titre reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hypochlorite (16,500 ppm available chlorine)</td>
<td>1 M or 2 M sodium hydroxide</td>
<td>aldehydes</td>
</tr>
<tr>
<td>Autoclaving at 121 °C after 1 M sodium hydroxide treatment</td>
<td>sodium dichloroisocyanurate (16,500 ppm available chlorine)</td>
<td>organic solvents</td>
</tr>
<tr>
<td>Autoclaving at 121 °C in 1 M sodium hydroxide</td>
<td>chaotropes (e.g. guanidine thiocyanate)</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
<td>Boiling in 1 M sodium hydroxide</td>
<td>95% formic acid</td>
<td>phenolic disinfectants</td>
</tr>
<tr>
<td></td>
<td>hot 1 M hydrochloric acid</td>
<td>chlorine dioxide</td>
</tr>
<tr>
<td></td>
<td>autoclaving for 18 min at 134–138 °C</td>
<td>iodine and iodates</td>
</tr>
<tr>
<td></td>
<td>autoclaving for 1 h at 132 °C</td>
<td>peracetic acid</td>
</tr>
<tr>
<td></td>
<td>autoclaving at 121 °C in 5% sodium dodecyl sulphate</td>
<td>protoclytic enzymes</td>
</tr>
<tr>
<td></td>
<td>dry heat at &gt; 200 °C</td>
<td>microwave irradiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV irradiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gamma irradiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>autoclaving after aldehyde, alcohol or dry heat treatments</td>
</tr>
</tbody>
</table>

## Hierarchy of Microbial Tolerance to Disinfection

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prions</td>
<td>Scrapie, Creutzfeld–Jakob disease, chronic wasting disease</td>
</tr>
<tr>
<td>Bacterial spores</td>
<td><em>Bacillus, Geobacillus, Clostridium</em></td>
</tr>
<tr>
<td>Protozoal oocysts</td>
<td><em>Cryptosporidium</em></td>
</tr>
<tr>
<td>Helminth eggs</td>
<td><em>Ascaris, Enterobius</em></td>
</tr>
<tr>
<td>Mycobacteria</td>
<td><em>Mycobacterium tuberculosis, M. terrae, M. chelonea</em></td>
</tr>
<tr>
<td>Small, non-enveloped viruses</td>
<td>Poliovirus, paroviruses, papilloma viruses</td>
</tr>
<tr>
<td>Protozoal cysts</td>
<td><em>Giardia, Acanthamoeba</em></td>
</tr>
<tr>
<td>Fungal spores</td>
<td><em>Aspergillus, Penicillium</em></td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td><em>Pseudomonas, Providencia, Escherichia</em></td>
</tr>
<tr>
<td>Vegetative fungi and algae</td>
<td><em>Aspergillus, Trichophyton, Candida, Chlamydomonas</em></td>
</tr>
<tr>
<td>Vegetative helminths and protozoa</td>
<td><em>Ascaris, Cryptosporidium, Giardia</em></td>
</tr>
<tr>
<td>Large, non-enveloped viruses</td>
<td>Adenoviruses, rotaviruses</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td><em>Staphylococcus, Streptococcus, Enterococcus</em></td>
</tr>
<tr>
<td>Enveloped viruses</td>
<td>Human immunodeficiency virus, hepatitis B virus, herpes simplex virus</td>
</tr>
</tbody>
</table>

Prions – Challenging Agents for the Reprocessing of Medical Devices

**CJD**

Incidence of sporadic CJD:
1-2 cases per 1 million persons per year.

Prions are essentially confined to the CNS.

**vCJD**

Prevalence of asymptomatic vCJD infections within the UK population:
One person in 2000 in the 1941-85 birth cohort.

Prions are present in the CNS and peripheral tissues.

<table>
<thead>
<tr>
<th>Table 1 Total cases of iatrogenic CJD world-wide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Neurosurgery</td>
</tr>
<tr>
<td>Depth electrodes</td>
</tr>
<tr>
<td>Corneal transplant</td>
</tr>
<tr>
<td>Dura mater</td>
</tr>
<tr>
<td>Human growth hormone</td>
</tr>
<tr>
<td>Human gonadotrophin</td>
</tr>
</tbody>
</table>

Iatrogenic Risks of CJD/vCJD Prompted Detailed Guidelines for Prion-Effective Reprocessing of Medical Devices

Germany

- Recommendations of Task Force vCJD

The routine reprocessing of medical devices not used on "risk patients" should include a minimum of two procedures that are at least partially effective against prions.
The distinct physico-chemical stability of prions suggest these pathogens as both:

- Test agents for the prescreening of candidate formulations in search of novel broad-range disinfectants.

- Bioindicators for the validation of heat-inactivation processes.
Conclusion (I): Dual Relevance of Prions with Regard to the Reprocessing of Medical Devices

- **Potential hazard**
  
  Iatrogenic transmission of prions
  
  - *Regulatory guidelines demand the demonstration of anti-prion efficacy of instrument reprocessing procedures.*

- **Potential use**
  
  Experimental utilization of prions
  
  - *Test agents for the development of novel broad-range disinfectants.*
  
  - *Bioindicators for the validation of heat-inactivation processes.*
How to Test Reprocessing Procedures for Efficacy Against Prions?

- **Steel Wire Assay** *(testing of cleaners and disinfectants)*
  - Contamination with prion brain homogenate → Drying over night → Washing: 5x in 45 ml A.dest. → Drying over night → Analysis of de-contamination


- **Tube Assay** *(testing of autoclaving procedures)*
  - Contamination with prion brain homogenate → Drying → Exposure to steam autoclaving procedures → Analysis of inactivation
Testing the Efficacy of Reprocessing Procedures Against Prion Infectivity by Bioassays in Animals

Bioassays in animals provide the gold standard for the detection and titration of prion infectivity, but they are

- time consuming
- expensive
- tightly limited in throughput
- ethically critical.

- Range of assay: $5.5 \log_{10}$ units (logs) or 9 logs of infectivity with reprocessed wires or autoclaved samples, respectively, for 263K scrapie prions.
Testing the Depletion of PrP<sup>TSE</sup> By Steel Wire Assay

- Range of assay with 263K scrapie prions: 3 log<sub>10</sub> units (logs) of PrP<sup>TSE</sup> depletion

- Range can be extended to 5 logs of PrP<sup>TSE</sup> depletion with steel wire grids as test carriers

Biochemical Detection of Prion Seeding Activity

Protein Misfolding Cyclic Amplification (PMCA)

Quantitative Serial Protein Misfolding Cyclic Amplification

PrP\textsuperscript{TSE}-Seed: Dried prion brain homogenate on tube- or wire surface

1:5 Dilution in normal brain homogenate

24 Cycles of incubation (1 h) and sonication (40 s)

PrPres-Amplificate

PMCA rounds

Western blot for PrPres detection after sample digestion with Proteinase K

Adapted from Lee & Caughey, 2007, Proc Natl Acad Sci USA, 104: 9551-9552
Testing the Efficacy of Cleaners and Disinfectants Against Prion Seeding Activity

*The infectivity on reference wires contaminated with 10^{-1}- to 10^{-8}-diluted scrapie brain homogenate had been determined by hamster bioassays in a previously published study

Pritzkow et al., 2011, PLOS ONE, 6: e20384
PMCA- and Hamster Assays Delivered Consistent Results for Prion Disinfection on Steel Wires

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Concentration</th>
<th>Time [min]</th>
<th>Temperature [°C]</th>
<th>Residual seeding activity on test wires (as compared to the seeding activity) on reference wires</th>
<th>Estimated residual infectivity per wire [LD_{50,c.imp.}]</th>
<th>Estimated reduction of infectivity</th>
<th>Residual infectivity per wire [LD_{50,c.imp.}]</th>
<th>Reduction of infectivity [logs]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>1.0 M</td>
<td>60</td>
<td>23</td>
<td>&lt; SA_{RW}(10^{-8})</td>
<td>&lt;3 × 10^{-2}</td>
<td>&gt;7</td>
<td>UD^{+}</td>
<td>≥5.5^{+}</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>2.5%</td>
<td>60</td>
<td>23</td>
<td>≤ SA_{RW}(10^{-8})</td>
<td>≤3 × 10^{-2}</td>
<td>≥7</td>
<td>UD^{+}</td>
<td>≥5.5^{+}</td>
</tr>
<tr>
<td>GdnSCN</td>
<td>4.0 M</td>
<td>10</td>
<td>23</td>
<td>≤ SA_{RW}(10^{-8})</td>
<td>≤3 × 10^{-2}</td>
<td>≥7</td>
<td>UD^{+}</td>
<td>≥5.5^{+}</td>
</tr>
<tr>
<td>SDS/NaOH</td>
<td>0.2%/0.3%</td>
<td>10</td>
<td>23</td>
<td>&lt; SA_{RW}(10^{-8})</td>
<td>&lt;3 × 10^{-2}</td>
<td>&gt;7</td>
<td>UD^{+}</td>
<td>≥5.5^{+}</td>
</tr>
<tr>
<td></td>
<td>0.2%/0.3%</td>
<td>5</td>
<td>23</td>
<td>≤ SA_{RW}(10^{-8})</td>
<td>≤3 × 10^{-2}</td>
<td>≥7</td>
<td>UD^{+}</td>
<td>≥5.5^{+}</td>
</tr>
<tr>
<td>n-Propanol</td>
<td>20%</td>
<td>10</td>
<td>23</td>
<td>&gt; SA_{RW}(10^{-8}) to ≤ SA_{RW}(10^{-7})</td>
<td>&gt;3 × 10^{-2} to &lt;3 × 10^{-1}</td>
<td>&gt;6 to ≤ 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline cleaner</td>
<td>1.0%</td>
<td>60</td>
<td>23</td>
<td>&lt; SA_{RW}(10^{-8})</td>
<td>&lt;3 × 10^{-2}</td>
<td>&gt;7</td>
<td>UD^{+}</td>
<td>≥5.5^{+}</td>
</tr>
<tr>
<td>Cidex OPA</td>
<td>0.55%</td>
<td>10</td>
<td>23</td>
<td>&gt; SA_{RW}(10^{-3}) to &lt; SA_{RW}(10^{-2})</td>
<td>&gt;3 × 10^{3} to &lt;3 × 10^{4}</td>
<td>&gt;1 to &lt;2</td>
<td>&gt;3 × 10^{3} to &lt;3 × 10^{4}</td>
<td>&gt;1 to &lt;2^{+}</td>
</tr>
<tr>
<td>Peracetic Acid</td>
<td>0.25%</td>
<td>60</td>
<td>23</td>
<td>&gt; SA_{RW}(10^{-2})</td>
<td>&gt;3 × 10^{4} to &lt;3 × 10^{5}</td>
<td>&gt;0 to &lt;1</td>
<td>&gt;3 × 10^{4} to ≤3 × 10^{5}</td>
<td>≥0 to &lt;1^{+}</td>
</tr>
</tbody>
</table>

Pritzkow et al., 2011, PLOS ONE, 6: e20384
Conclusion (II): Seeding Activity and Infectivity of 263K Scrapie Prions

- The biochemical seeding activity of PrP\textsuperscript{TSE} on tube- or steel wire surfaces contaminated with 263K scrapie prions could be titrated by quantitative PMCA.

- The biochemical seeding activity of PrP\textsuperscript{TSE} and the biological infectivity of 263K scrapie prions could be translated into each other on the basis of a consistent quantitative correlation.
Testing the Efficacy of Autoclaving Procedures Against Prion Seeding Activity

- Sterilization of 263K scrapie hamster brain homogenate (1 mg of tissue, 100 μg total protein)

- 134°C at retention times of 0, 3 or 5 min

- Reduction of seeding activity: > 7 logs

Reference samples [amount of 263K scrapie brain tissue]
Testing the Efficacy of Reprocessing Procedures Against Prion Infectivity by Cell Assays

- Primary hamster glia cultures

<table>
<thead>
<tr>
<th>P</th>
<th>0</th>
<th>2.5 x 10^{-5}</th>
<th>1 x 10^{-6}</th>
<th>1 x 10^{-7}</th>
<th>1 x 10^{-8} [g / culture]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>42</td>
<td>3</td>
<td>40</td>
<td>3</td>
<td>40 [DPE]</td>
</tr>
</tbody>
</table>

Accumulation of PrPres in hamster glia cultures exposed to the indicated amounts of homogenized 263K scrapie hamster brain tissue

Pritzkow et al., 2011, PLOS ONE, 6: e20384
Conclusion (III): In Vitro Assays Facilitate the Analysis and Utilization of Prions

In vitro assays for PrP\text{\textsc{tse}} depletion, prion seeding activity and prion infectivity

- are faster, cheaper, ethically less critical and partly more sensitive than bioassay titrations of prions in animals,
- open the perspective to substantially reduce - or even replace - animal bioassays in prion disinfection studies,
- can practically harness prions as informative test pathogens for the development of novel broad-range disinfectants and as bioindicators for the validation of heat inactivation procedures.
Using Prions as Test Agents in Search of New Broad-Range Disinfectants

Test schedule for candidate formulations

- Test the depletion of PrP\textsuperscript{TSE}
- Test the reduction of prion seeding activity
- Test the reduction of prion infectivity
- Test the efficacy against bacteria, viruses and fungi
- Validate the reduction of prion infectivity in animals

<table>
<thead>
<tr>
<th>In vitro-assays</th>
<th>In vivo-assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrP\textsuperscript{TSE} depletion</td>
<td>prion infectivity validation</td>
</tr>
<tr>
<td>prion seeding activity reduction</td>
<td>disinfector efficacy against microorganisms</td>
</tr>
<tr>
<td>prion infectivity reduction</td>
<td>prion infectivity reduction in animal models</td>
</tr>
</tbody>
</table>
Fast, Broad-Range Disinfection of Bacteria, Viruses, Fungi and Prions

Titre reductions with
0.2% SDS / 0.3% NaOH / 20% n-Propanol (20 min, RT, pH 13)

- **Prions**  
  (263K Scrapie – Agent on Steel Wires) ≥ 5 logs

- **Bakteria / Mycobacteria**  
  *(E. faecium, M. avium)* ≥ 6 logs

- **Viruses**  
  (Poliovirus and Hepatitis A – Virus on Steel Wires with Coagulated Blood) ≥ 4 logs

- **Fungi**  
  *(Spores of Aspergillus niger)* ≥ 5 logs

Beekes et al. (2010), J Gen Virol, 91: 580-589
**Mildly Alkaline Disinfectant Formulation: PrP\textsuperscript{TSE}-Depletion Assay**

2.5% K\textsubscript{2}CO\textsubscript{3} (containing sarcosyl, urea, EDTA, n-propanol):

1h, 55°C, pH 10.5

- Reduction of PrP\textsuperscript{TSE}: ~5 logs\(^*\) (*test limit)

- Similar result with pH 11.5 at RT for 1h

Dilutions of eluates from reference steel wire grids that had been contaminated with 10% 263K scrapie brain homogenate (SBH)

Reprocessed test grids (originally contaminated with 10% 263K SBH)
Mildly Alkaline Disinfectant Formulation: Seeding Activity Assay

2.5% $\text{K}_2\text{CO}_3$
(containing sarcosyl, urea, EDTA, n-propanol)

Reprocessed steel wires originally contaminated with $10^{-1}$-diluted 263K scrapie brain homogenate (SBH)

- **Reduction of seeding activity:** $> 7$ logs

<table>
<thead>
<tr>
<th></th>
<th>PMCA rounds</th>
<th>PMCA rounds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P</strong></td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>1 h, 55°C, pH 10.5</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td>1 h, RT, pH 11.5</td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
</tbody>
</table>

Reference steel wires contaminated with the indicated dilutions of 263K SBH
Mildly Alkaline Disinfectant Formulation: Microbiological Assays

- 2.5% $K_2CO_3$ containing sarcosyl, urea, EDTA and n-propanol (1 h at pH 10.5 and 55°C or at pH 11.5 and RT)

  has similar broad-range efficacy against bacteria, viruses and fungi as

- 0.2% SDS / 0.3% NaOH / 20% n-propanol (20 min at pH 13 and RT)
Mildly Alkaline Disinfectant Formulation: Further Testing

- Cell assay for prion infectivity
- Animal bioassay for prion infectivity
- Carrier assay for unspecific protein fixation

Beekes et al., 2010, J Gen Virol, 91: 580-589
Recent reports suggested that the disinfection of microbial pathogens and prions can be smoothly combined for the routine reprocessing of medical instruments.

Mildly alkaline K$_2$CO$_3$-based formulations may be also suitable for this purpose at pH 10.5 (55°C) or 11.5 (RT).
Seeding Active Protein Particles in Other Neurodegenerative Diseases

<table>
<thead>
<tr>
<th>Native Protein</th>
<th>Aggregated Protein or Peptide</th>
<th>Main Associated Diseases in humans</th>
<th>Acquired by Infection in Humans</th>
<th>Seeded Aggregation in Cell Culture</th>
<th>Seeded Aggregation In Vivo</th>
<th>Cell-to-cell Spread in Cell Culture</th>
<th>Cell-to-cell Spread In Vivo</th>
<th>Tissue Migration In Vivo</th>
<th>Inducible Clinical Disease in Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrP&lt;sup&gt;G&lt;/sup&gt;</td>
<td>PrP&lt;sup&gt;Sc&lt;/sup&gt;</td>
<td>(variant, intragenic) Creutzfeldt-Jacob, Kuru (sporadic, familial) Creutzfeldt-Jacob, Fatal familial insomnia, Gerstmann-Straussler Scheinker</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Tau</td>
<td>Tau</td>
<td>frontotemporal lobar dementia, Alzheimer</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>n.d.</td>
</tr>
<tr>
<td>α-synuclein</td>
<td>α-synuclein</td>
<td>Parkinson, Lewy body dementia</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>n.d.</td>
<td>yes (acceleration in mutant mice)</td>
</tr>
<tr>
<td>APP</td>
<td>β-amyloid</td>
<td>Alzheimer</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

Modified from: Polymenidou & Cleveland, 2011, Cell, 147: 498-508
Reprocessing by anti-prion effective procedures seems likely to decontaminate medical devices also from other protein seeds.

However, theoretically Aβ-, Tau- or α-Synuclein seeds may be more tolerant to cleaning- or inactivation procedures than prions.
Depletion of Aggregated α-Synuclein on Steel Wires Grids

- Reduction of aggregated α-Synuclein: ~ 500-fold* (*test limit)

Western blot with mAb LB509
Prions – Challenging Agents and Useful Markers for the Cleaning and Disinfection of Medical Devices

- Effective against bacteria, viruses, fungi, prions (and other protein seeds)
- Material- and device compatible
- Routinely usable
- Free of protein fixating effects
- Cost-efficient
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