Prion inactivation

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The nature of the infectious agent

- It is the abnormal isoform ($\PrP^{\text{sc}}$) of a host cellular protein called prion protein ($\PrP^{\text{c}}$)

- The $\PrP^{\text{sc}}$ is characterized by an increased number of $\beta$-pleated sheets whereas the $\alpha$-helical content of the protein is decreased, resulting in a different conformational arrangement than that of the $\PrP^{\text{c}}$ and profound changes in properties of the protein

- The $\PrP^{\text{c}}$ is susceptible to proteases, whereas the $\PrP^{\text{sc}}$ is partially resistant

- The pathogenic prion accumulates in neural cells, disrupting function and leading to vacuolization and cell death. Diseases caused by prions are called Transmissible Spongiform Encephalopathies (TSEs)
Animal TSEs

- Scrapie
- Bovine spongiform encephalopathy (BSE)
- Chronic wasting disease of mule, deer and elk (CWD)
- Transmissible mink encephalopathy (TME)
- Feline spongiform encephalopathy (FSE)
- Spongiform encephalopathies of captive zoo animals
Human TSEs

- Kuru
- Creutzfeldt-Jakob disease (CJD)
  - sporadic
  - familial
  - iatrogenic
  - variant (vCJD)
- Fatal insomnia
- Gerstmann-Straussler-Scheinker disease (GSS)
Infectivity of tissues

- High risk of infection carry the brain (including dura mater), the spinal cord, and the eye (e.g. corneas) 
  
  *W.H.O, 2003*

- Prions are systematically detected in the peripheral tissues (lymphoreticular system and blood) of patients with vCJD
  
  *Wadsworth and Collinge 2007*

- There may be a risk of iatrogenic vCJD transmission via blood and contaminated surgical and medical instruments, including endoscopes and cerebral electrodes
  
  *Joiner et al., 2005*
Decontamination and inactivation procedures for pathogenic prions
General considerations

- Prions are highly resistant to disinfection and sterilization by most of the physical and chemical methods in common use for decontamination of infectious pathogens.
- Prion infectivity is strongly stabilized by drying or fixation with alcohol, formalin or glutaraldehyde.
- Contaminated materials should not be exposed to fixation reagents and should be kept wet between the time of use and disinfection, by immersion in chemical disinfectants (or even water).
- The likelihood of prion inactivation depends on the prion strain, the nature of the surface concerned and the inoculum.
General recommendations for decontamination of instruments

- It should be taken into account the infectivity level of the tissue contaminating the instrument.
- The safest and most unambiguous method is to discard and destroy the used instruments by incineration.
- **Mechanical cleaning** of surgical instruments that are going to be reused should be carried out in advance of decontamination.
- **Two or more different methods** of prion inactivation should be combined in any sterilization procedure.
Cleaning procedure

- Those devices that are impossible or difficult to clean could be discarded.
- Alternatively, one should place the contaminated items in a container filled with a liquid (e.g., saline, water, or phenolic solution) to retard adherence of prion protein to the medical device.
- The instruments should be kept moist until cleaned and decontaminated.
- Flash sterilization should not be used for reprocessing (i.e., steam sterilization of an unwrapped item for 3 min at 132°C).
Low pressure plasma technique for cleaning of instruments

- A plasma is a partially ionized gas
- The process acts under vacuum conditions
- The gas (oxygen and argon) is ionized with the help of a high frequency generator
- The formed reactive particles react in a direct way with prion proteins (organic material) eliminating them from instrument surfaces
- **Advantages:** no toxic residue effects, reduced turnover time, and applicability for cleaning of heat and moisture-sensitive instruments

*Whittaker et al., 2004*
Autoclave/chemical methods for heat-resistant instruments (in order of decreasing effectiveness)
• **Immerse** in **NaOH** (1N) and **heat** in a gravity displacement autoclave at 121°C for 30 min; clean; rinse in water and subject to routine sterilization

• **Spills, corrosion and explosion** have been reported with this technique!
• Immerse in NaOH (1N) or 5.25% sodium hypochlorite (25.000 ppm available chlorine) for 1 hour; transfer instruments to water; heat in a gravity displacement autoclave at 121°C for 1 hour; clean; and subject to routine sterilization
• Immerse in NaOH (1N) or sodium hypochlorite for 1 hour; remove and rinse in water, then transfer to an open pan and heat in a gravity displacement (121°C) or porous load (134°C) autoclave for 1 hour; clean; and subject to routine sterilization
• Immerse in \textbf{NaOH (1N)} and \textbf{boil} for 10 min at atmospheric pressure; clean; rinse in water and subject to routine sterilization
• **Immerse** in **sodium hypochlorite** (preferred) or **NaOH (1N)** (alternative) at ambient temperature for 1 hour; clean; rinse in water and subject to routine sterilization
• Autoclave at $134^0C$ for 18 minutes

• In worse-case scenarios (brain tissue bake-dried onto the instruments) infectivity will be largely but not completely removed!

• The temperature should not exceed $134^0C$, because the effectiveness of autoclaving may decline as the temperature is increased!
Cautions regarding the use of NaOH and NaOCl

- In principle, NaOH does not corrode stainless steel. However, some formulations of stainless steel can be damaged (including some used for surgical instruments)
- NaOH is corrosive to glass and aluminum, whereas NaOCl is not
- NaOCl is corrosive to both stainless steel and autoclaves and cannot be used as an instrument bath in the autoclave
- If NaOCl is used to clean or soak an instrument, it must be completely rinsed from the surfaces before autoclaving!
Chemical methods for surfaces and heat-sensitive instruments
Before prion inactivation...

- Minimize environmental contamination with prions, by using **disposable cover sheets** on work surfaces.
- **Disposable** protective equipment **covers** should be placed on powered instruments (e.g., drills), endoscopes, microscopes and other complex and expensive instruments.
- Contaminated environmental surfaces should be cleaned first and then spot-decontaminated.
Decontamination methods
• Flood with NaOH (2N) or undiluted sodium hypochlorite; let stand for 1 hour; mop up and rinse with water
Where surfaces or instruments can not tolerate NaOH or sodium hypochlorite, clean and then use one of the following partially effective (against prions) chemical disinfectants:

1. chlorine dioxide
2. glutaraldehyde
3. guanidinium thiocyanate (4 mol/L)
4. iodophores
5. sodium dichloro-isocyanurate
6. sodium metaperiodate
7. urea (6 mol/L)
8. combination of Cu (CuSO$_4$ 500 μmol/L) and peracetic acid (5%)

(Lehmann et al., 2009)
For complex and expensive instruments...

- Those parts of the device that come into contact with internal tissues of patients, should be subjected to the most effective decontamination procedure that can be tolerated by the instrument.
- Some instruments can be partly disassembled (e.g., drills and drill bits). Removable parts that would not be damaged by autoclaving, NaOH or bleach should be dismounted and treated with these agents.
- The manufacturer of the instrument should be consulted with.
Autoclave/chemical methods for dry goods
• **Small** dry goods that can withstand either NaOH or sodium hypochlorite should first be immersed in one or the other solution and then heated in a porous load autoclave at $\geq 121^0\text{C}$ for 1 hour

• **Bulky** dry goods or dry goods of any size that cannot withstand exposure to NaOH or sodium hypochlorite should be heated in a porous load autoclave at $134^0\text{C}$ for 1 hour
Decontamination of wastes and waste-contaminated materials.
- All waste liquids and solids must be captured and treated as infectious waste
- Liquids used for cleaning should be decontaminated in situ by addition of NaOH or NaOCl; be sterilized and may then be disposed of as routine hospital waste
- Absorbents (e.g., sawdust) may be used to stabilize liquids that will be transported to an incinerator; however, this should be added after decontamination
- Great care is required in the use of brushes, scouring tools, toweling and tools used for disassembling contaminated apparatus. They should either be disposable or selected for their ability to withstand the prion inactivation procedures
Promising prion inactivation procedures
Hydrogen peroxide gas plasma sterilization

- The precise mode of action of H$_2$O$_2$ against prions remains to be clarified
- Instruments are placed in H$_2$O$_2$ (90%), low temperature (53°C), gas plasma sterilizers for 7 minutes
- A significant reduction in prion infectivity ($\geq$ 5 to 6 log) has been observed for 3 prion strains (hamster-adapted scrapie 263K, mouse-adapted BSE 6PB1, and a vCJD)

Roger-Kreuz et al., 2009
Photo-Fenton reagent

- Fenton reagent is a mixture of iron salts and $\text{H}_2\text{O}_2$.
- It is an oxidative system, which produces hydroxide (OH) radicals.
- The reaction can be greatly enhanced by UV/visible light (artificial or natural) producing additional OH radicals and leading to the regeneration of the catalyst.
- The photocatalytic treatment of contaminated instruments causes not only the elimination of prions but of the entire protein load.

Paspaltsis et al., 2009
Genetically engineered protease (MC3)

- MC3 is an alkaline protease and represents a genetically engineered variant of the *Bacillus lentus* subtilisin
- It presents improved stability and catalytic properties at alkaline pH, digesting prion material
- It can reduce BSE301V infectivity to > 7 log
  
  *Dickinson et al., 2009*

- SEAC recommends that any decontamination process for prions should demonstrate a reduction in infectivity of ≥ 5 log

*October 9th, 2009*
Thank you very much for your attention!