

# Fundamentals of prions and their inactivation (Review)

AKIKAZU SAKUDO<sup>1</sup>, YASUHISA ANO<sup>2</sup>, TAKASHI ONODERA<sup>3</sup>, KAYAKO NITTA<sup>4</sup>,  
HIDEHARU SHINTANI<sup>5</sup>, KAZUYOSHI IKUTA<sup>6</sup> and YASU HARU TANAKA<sup>1</sup>

<sup>1</sup>Laboratory of Biometabolic Chemistry, School of Health Sciences, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215; <sup>2</sup>Central Laboratories for Frontier Technology, Kirin Holdings Co., Ltd., 1-13-5 Fukuura Kanazawa-ku, Yokohama-shi, Kanagawa 236-0004; <sup>3</sup>Department of Molecular Immunology, School of Agricultural and Life Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113-8657; <sup>4</sup>Urasoe General Hospital, 4-16-1 Iso, Urasoe, Okinawa 901-2132; <sup>5</sup>Faculty of Science and Engineering, Chuo University, 1-13-27 Kasuga, Bunkyo-ku, Tokyo 112-8551; <sup>6</sup>Department of Virology, Center for Infectious Disease Control, Research Institute for Microbial Diseases, Osaka University, Yamadaoka, Suita, Osaka 565-0871, Japan

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**Abstract.** Prion is an infectious particle composed of an abnormal isoform of the prion protein (PrP<sup>Sc</sup>) and causes prion diseases such as bovine spongiform encephalopathy (BSE), Creutzfeldt-Jakob disease (CJD) and scrapie. Host cells express cellular prion protein (PrP<sup>C</sup>), which plays roles in normal functions such as anti-oxidative stress. PrP<sup>Sc</sup> is derived from PrP<sup>C</sup> and produced by conformational conversion. Prion is notorious as a resistant pathogen, being difficult to inactivate with conventional sterilization procedures. Therefore, to prevent prion-caused iatrogenic diseases, the use of appropriate procedures to inactivate prions is important. For examples, alcohol treatment, autoclave (121°C, 20 min) and  $\gamma$ -ray irradiation, which are used for disinfection, antisepsis or sterilization of viruses and bacteria, are not effective against prion. This is a fundamental review of prions and methods of their inactivation.

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## 1. Pathogens and prions

Pathogens can be transmitted through food, air, drinking water, body fluids and attachment (1). There are many types,

including protozoa, helminths, prions, viruses, fungi, algae, mycobacteria, bacteria, viroids and bacteriophages (Fig. 1), but relatively few are directly connected to diseases. In most cases of pathogen-related diseases, the causes are not fully taken into account or sporadic, and not reported to any official agency because they are not severe or cultures are never obtained (2,3). Some pathogens change their forms under some circumstances. Certain bacteria and fungi form spores under low-nutrient or dry conditions. Protozoa form oocysts and cysts dependent on their life cycle, while helminths form eggs. After an infection, viruses inject their genome into host cells. After producing viral proteins using the host cell's machinery, the virus matures. Among viruses, there are many types such as i) bacteriophages, which infect bacteria, ii) viroids, which are only RNA, devoid of proteins and infect higher plants causing crop diseases and iii) animal viruses. Animal viruses are divided into two groups; non-enveloped and enveloped (1). Besides viruses, prions (proteinaceous infectious particles) (4) have been studied in the field of virology, because they cause prion diseases, which have been classified as slow viral diseases. Prion is important, because it is the most difficult pathogen to inactivate and transmissible by eating, transfusion, and neurosurgery.

Prion diseases are neurodegenerative disorders caused by protein-based infectious particles (prions). Main component of prion is an abnormal isoform of the prion protein (PrP<sup>Sc</sup>) (5). Recently, the emergence of bovine spongiform encephalopathy (BSE) and its variant Creutzfeldt-Jakob disease (vCJD) has been highlighted. The issue has enhanced concern over food safety, safe transfusions, and safe neurosurgery. However, with the recent emergence of avian and swine influenza viruses (6), interest in BSE and vCJD has waned and information on prion diseases is still limited, especially notable in the areas of therapy and pre-mortem diagnosis.

The history of prion research is relatively long, with Cullie and Chelle reporting scrapie to be an infectious disease in 1936 (7). Although scrapie had been thought to be caused by a virus and classified as a slow viral disease, Prusiner proposed a new type of pathogen composed only of

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*Correspondence to:* Dr Akikazu Sakudo, Laboratory of Biometabolic Chemistry, School of Health Sciences, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan  
E-mail: sakudo@med.u-ryukyu.ac.jp

**Key words:** prion protein, sterilization, gas plasma

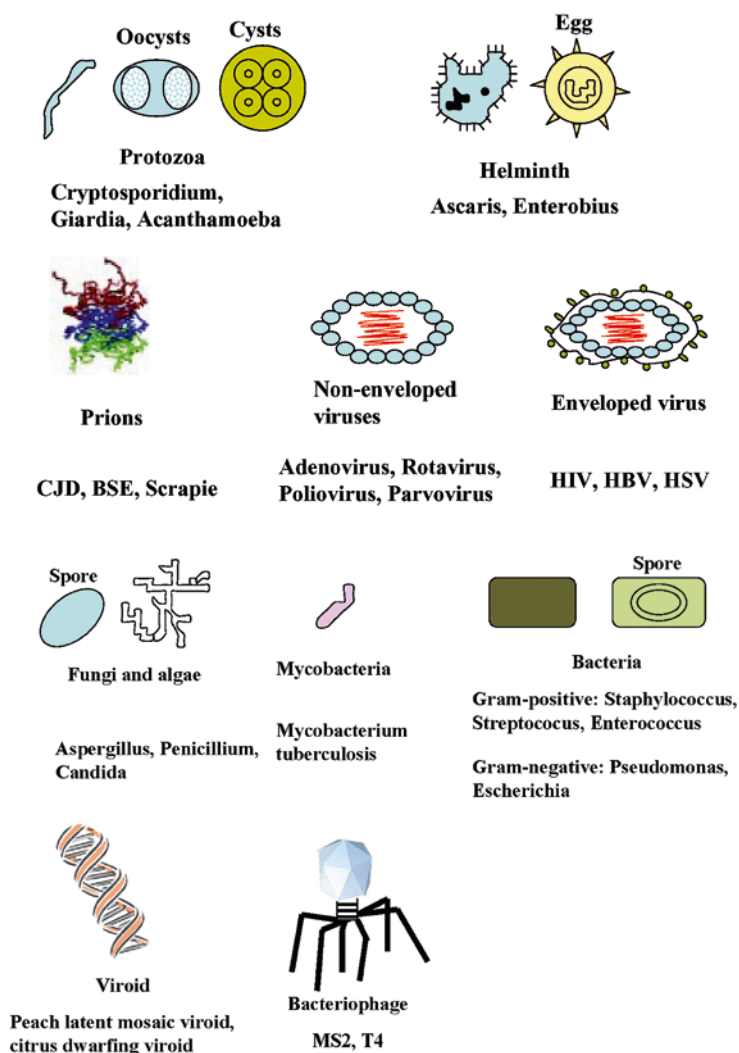


Figure 1. Features of pathogens. There are various types of pathogens including protozoa, helminths, prions, viruses, fungi, algae, mycobacteria, bacteria, bacteriophages and viroids. Some pathogens change form dependent on circumstances. Some bacteria form spores under low-nutrient conditions. Fungi makes spores under dry conditions. Styles of protozoa change to oocysts and cysts with growth depending on the life cycle, while those of helminths also form eggs. Methods of detecting pathogens are closely related to the composition of pathogens. Nucleic acid-based methods are commonly used, because the genomes of all pathogens except prions are composed of nucleic acid (DNA or RNA). As prion is thought to only be composed of proteins, nucleic acid-based methods are not applicable. HIV, human immunodeficiency virus; HBV hepatitis B virus; HSV, herpes simplex virus; CJD, Creutzfeldt-Jakob disease; BSE, bovine spongiform encephalopathy. Modified from Fig. 2 in Sakudo *et al* (49) with permission from NOVA Science Publishers.

protein (4). Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler-Scheinker disease (GSS) has been known for some time, with case reports by Creutzfeldt in 1920 (8), Jakob in 1921 (9), and Gerstmann *et al* in 1936 (10). The basic feature of prion disease as a transmissible spongiform encephalopathy became clear from findings for Kuru disease, which occurs in the Fore linguistic group in the Eastern Highlands of Papua New Guinea, who have a custom of eating human brain as part of mortuary rites (11). Transmission was validated by the finding that brain homogenate containing the Kuru agent caused similar symptoms to Kuru in monkeys following an intracerebral injection (11). Meanwhile, the causes of human prion diseases vary, i.e.: infection for Kuru and iatrogenic CJD (iCJD), mutation of the prion protein (PrP) gene for GSS, fatal familial insomnia (FFI) and familial CJD (fCJD), and unknown cause for sporadic CJD (sCJD) (5) (Table I). Generally, the prion diseases caused by mutation of the PrP

Table I. Human prion diseases.

Disease	Cause
Kuru	Infection
iCJD	Infection
sCJD	Unknown
fCJD	Mutation of PrP gene
vCJD	Infection
GSS	Mutation of PrP gene
FFI	Mutation of PrP gene

CJD, Creutzfeldt-Jakob disease; iCJD, iatrogenic CJD; sCJD, sporadic CJD; fCJD, familial CJD; vCJD, variant CJD; GSS, Gerstmann-Sträussler-Scheinker Syndrome; FFI, fatal familial insomnia; PrP, prion protein.

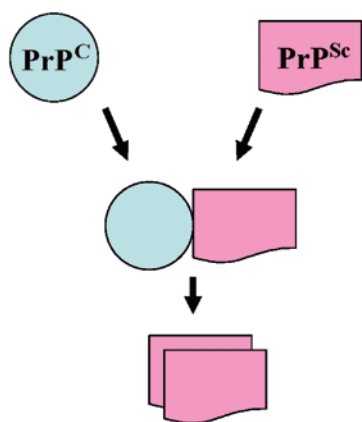


Figure 2. Conformational change of the cellular isoform of prion protein ( $\text{PrP}^{\text{C}}$ ) to an abnormal isoform of prion protein ( $\text{PrP}^{\text{Sc}}$ ). After infection,  $\text{PrP}^{\text{C}}$  is converted into  $\text{PrP}^{\text{Sc}}$ .  $\text{PrP}^{\text{Sc}}$  aggregates, inducing the deposition of prion protein (PrP) in the brain. The accumulation causes the death of neurons. In addition, loss of  $\text{PrP}^{\text{C}}$  may contribute to the pathogenesis, because  $\text{PrP}^{\text{C}}$  plays a role in anti-oxidative stress, calcium homeostasis, circadian rhythms, spatial learning, and memory.

gene are relatively less infectious. These diseases have different symptoms, because the areas of the brain affected differ: for example, the cerebral cortex in CJD, the cerebellum in GSS and thalamus in FFI. Such damage is caused by neuronal cell loss, astrogliosis, and vacuolation, leading to degeneration (5). Another prion disease, vCJD, is important in connection to BSE, which affected as many as 180,000 cattle from 1985 to 1995 in the United Kingdom (UK) (12). The total number of cattle with BSE in the UK is about 184,500, whereas the total number in other countries is about 5,800 (13). Therefore, >95% of BSE cases have occurred in the UK. The problem is that BSE prion is a causative agent of vCJD (14,15). Meanwhile, the cause of most prion diseases (85-90%) remains unclear. Such prion diseases are known as sporadic CJD (sCJD).

## 2. Methods for detecting prions

After an infection, cellular PrP ( $\text{PrP}^{\text{C}}$ ), which is expressed in host cells, especially neurons, and has possible functions including the regulation of anti-oxidative stress, calcium and copper homeostasis, circadian rhythms, spatial learning, memory, signalling, and synapse formation (16,17), changes into  $\text{PrP}^{\text{Sc}}$  (Fig. 2).  $\text{PrP}^{\text{Sc}}$  accumulates in the brain and forms PrP deposits, which are protease-resistant (Table II). The accumulation causes neuronal cell loss, astrogliosis, and vacuolation, leading to spongiform and eventually death (18). Prion diseases, unlike other amyloid diseases, are transmissible. Generally, transmission is more difficult between different species than between identical species, a phenomenon known as the 'species barrier' (19). However, BSE can be transmitted to humans, resulting in vCJD (14), whereas epidemiological data suggest that scrapie cannot directly infect humans. Therefore, the study of BSE is important in terms of food hygiene (20). In Japan, all cows over 21 months old are checked for BSE (21). The method is as follows (Fig. 3): a homogenate is prepared from the brain obex and treated with proteinase K (PK). The treated sample is applied to a microtiter plate for

Table II. Characteristics of  $\text{PrP}^{\text{C}}$  and  $\text{PrP}^{\text{Sc}}$ .

	$\text{PrP}^{\text{C}}$	$\text{PrP}^{\text{Sc}}$
Resistance to protease	Low	High
Distribution in cell	Cell surface	Intracellular
GPI anchor	Present	Present
Release from cell surface by PIPLC	Positive	Negative
Half life	3-6 h	>24 h
Solubility	High	Low
Conformation	42% $\alpha$ -helix, 3% $\beta$ -sheet	30% $\alpha$ -helix, 43% $\beta$ -sheet
Infectivity	Negative	Positive

$\text{PrP}^{\text{C}}$ , cellular isoform of prion protein;  $\text{PrP}^{\text{Sc}}$ , abnormal isoform of prion protein; GPI, glycosylphosphatidyl inositol; PIPLC, phosphatidyl-inositol specific phospholipase C.

absorption then detected with anti-PrP antibody. If the result is positive, the above-mentioned enzyme-linked immunosorbent assay (ELISA) is performed repeatedly. If a positive result is obtained again, Western blotting and immunohistochemistry (IHC) are performed (Fig. 4). The Western blotting uses a membrane to absorb PK-treated proteins separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). After the absorption, PK-resistant PrP ( $\text{PrP}^{\text{res}}$ ) in the membrane-bound proteins is detected with anti-PrP antibody. Importantly, Western blotting provides information on not only prion infections but also the mobility of peptides, which is influenced by the host genotype and prion strains (22,23). As shown in Fig. 4B, the IHC is based on representative pathological features of prion diseases including neuronal cell loss, vacuolation, astrogliosis and amyloid plaques. In the IHC analysis of brain sections, the accumulation of PrP amyloid plaques, neuronal cell loss (or vacuolation) and astrogliosis are examined by light microscopy. Although vacuolation is also used as an index of prion infection, various combinations of prion strains with host species result in the accumulation of PrP without vacuolation in brain sections (24,25). Recently, the protein misfolding cyclic amplification (PMCA) method has been developed (26), which enables *in vitro* amplification of  $\text{PrP}^{\text{res}}$  from a small quantity of  $\text{PrP}^{\text{Sc}}$  as seed by sequential cycles of incubation and sonication. Interestingly, levels of  $\text{PrP}^{\text{res}}$  amplified by this method correlated with the prion infectivity titer (27). Furthermore, PMCA could detect prions in blood (28). In addition, it could be used to diagnose not only terminally diseased hamsters but also prion-infected pre-symptomatic hamsters (29). This method has the highest sensitivity of any method for detecting  $\text{PrP}^{\text{res}}$  reported so far. To date, its application to sheep (28), goat (30), cattle (30), sCJD (30), vCJD (31), CWD (32) and hamster scrapie (26) and mouse scrapie (33) has been reported. Recently, PMCA has been modified, leading to recombinant PrP-PMCA (rPrP-PMCA) (34) and its combination with the quaking-induced conversion (QUIC) reaction (34,35). In addition, information obtained from PMCA will contribute to understanding the

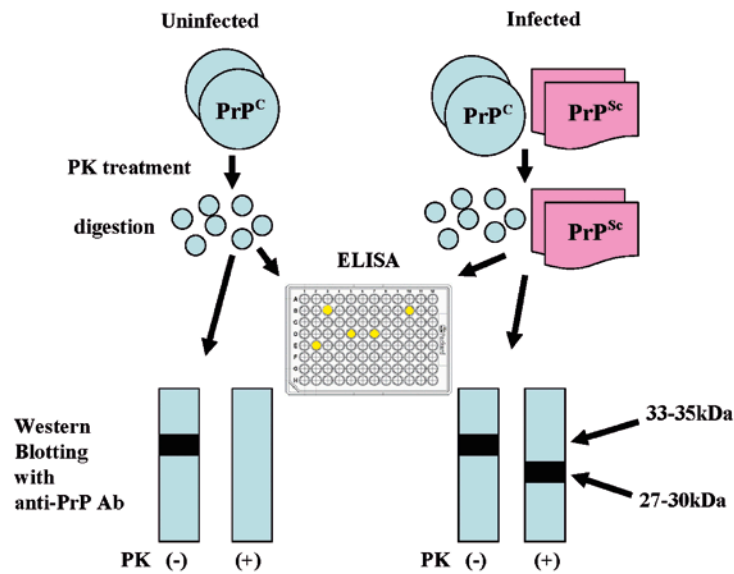


Figure 3. Diagnostic methods for prion infections. For the diagnosis of prion diseases, brain tissue is used. Therefore, the diagnosis is post-mortem. Most methods of diagnosing prion diseases are based on the characteristics of PrP<sup>Sc</sup>, which is resistant to proteinase K (PK). PK completely degrades PrP<sup>C</sup> but only partially digests PrP<sup>Sc</sup>, because PrP<sup>Sc</sup> makes aggregates and those cores remain. After the treatment, Western blotting and enzyme-linked immunosorbent assay (ELISA) with anti-PrP antibody detect PK-resistant PrP, when PrP<sup>Sc</sup> is included in the sample. As the N-terminal region of PrP<sup>Sc</sup> is digested with PK(+), PK-resistant PrP (27-30 kDa) shows a shift to a lower molecular weight compared to untreated PrP(-) (33-35 kDa) in Western blotting.

mechanism of conformational change of PrP. So far, several factors such as RNA and metals have been found to be involved in the conformational change using PMCA (36,37). Furthermore, *in vivo* analyses of the use of such factors may provide clues to the treatment of prion diseases.

### 3. Iatrogenic prion disease and prion inactivation

Although 4 cases of iCJD caused by neurosurgery have been reported, all are from the 1980s or earlier. Therefore, a causal relationship remains to be determined (38). However, difficulty in the inactivation of prions requires appropriate treatment of the surgical instruments used for craniotomy, spinal surgery, ophthalmologic surgery, etc. The most important difference between prion and other pathogens is that prion has no nucleic acid (5). Therefore, it cannot be inactivated by conventional sterilization procedures such as autoclaving (121°C, 20 min), exposure to UV and γ-ray irradiation (5). Although alcohol treatments such as 70% ethanol are effective for bacteria and enveloped viruses, they have no effect on prions. To inactivate prions, use of an autoclave under severe condition (134°C, 18 min), NaOH (1 N, 20°C, 1 h), SDS (30%, 100°C, 10 min), and NaOCl (20000 ppm, 20°C, 1 min) is recommended (Table III). As a practical method for prion inactivation, the following procedures are recommended (38). i) Washing with appropriate detergents + SDS treatment (3%, 3-5 min), ii) treatment with alkaline detergents (80-93°C, 3-10 min) + autoclaving (134°C, 8-10 min), iii) washing with appropriate detergents + autoclaving (134°C, 18 min) and iv) washing with alkaline detergents (at a concentration and temperature according to instructions) + vaporized hydrogen peroxide gas plasma sterilization. The most important point is that a dried prion-infected apparatus is difficult to be sterilized. Therefore, after use of the apparatus, prompt washing is essential (38).

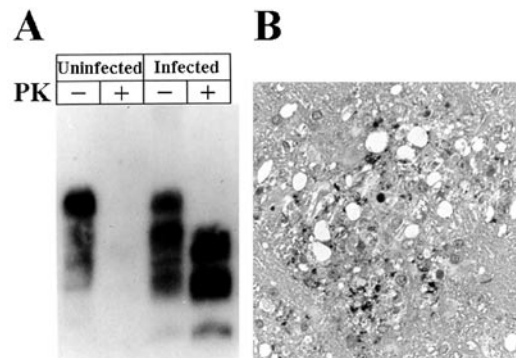


Figure 4. Western blotting and immunohistochemistry (IHC). (A) PrP from brain membranous fractions of uninfected mice (uninfected) and terminally diseased mice intracerebrally inoculated with Obihiro-1 prions (infected) was detected by Western blotting with anti-PrP antibody, 6H4 and horseradish peroxidase (HRP)-conjugated anti-mouse IgG. Each fraction was treated (+) or not (-) with PK. Modified from Fig. 1 in Inoue *et al* (50) with permission from the National Institute of Infectious Diseases. (B) Immunohistochemistry of the brains of terminally prion-diseased mice infected with Obihiro-1 prions. PrP was detected by an anti-PrP antibody, 6H4. Vacuolation and PrP deposits were seen. Courtesy of Dr Yuji Inoue (Department of Virology, Center for Infectious Disease Control, Research Institute for Microbial Diseases, Osaka University, Japan).

Then, autoclaving at 134°C and vaporized hydrogen peroxide gas plasma sterilization, etc. should be performed for prion inactivation.

Finally, we focus on gas plasma sterilization. Although not as well known as major sterilization procedures like autoclaving, there are various reports on prion inactivation using this method (39). Gas plasma is the fourth state of matter after solid, liquid and gas (40). Gas plasma is generated by removing electrons and producing a highly excited mixture of charged

Table III. Effective treatment on inactivation of prion.

Treatment	Refs.
NaOCl (20000 ppm, 20°C, 1 h)	(39)
NaOH (1 N, 20°C, 1 h)	(39)
Autoclave under soaked condition in water (134°C, 18 min)	(39)
Alkaline detergent (1.6%, 43°C, 15 min)	(39)
Phenolic disinfectant (5%, 20°C, 30 min)	(39)
3% SDS, 100°C, 10 min	(51)
7 M guanidine hydrochloride (room temperature, 2 h)	(51)
3 M guanidine thiocyanate (room temperature, 2 h)	(51)
3 M trichloroacetic acid (room temperature, 2 h)	(51)
60% formic acid (room temperature, 2 h)	(51)
50% phenol (room temperature, 2 h)	(51)
Enzymatic detergent (0.8%, 43°C, 5 min) + hydrogen peroxide gas plasma sterilization (1.5 mg/l, 25°C, 3 h)	(39)
Vaporized hydrogen peroxide (2 mg/l, 30°C, 3 cycles)	(39)

\*Autoclaving with no soaking in water is insufficient for prion inactivation (dry conditions cause difficulty in inactivation) (39,52). \*Enzymatic detergent (0.8%, 43°C, 5 min) + autoclave (121°C, 20 min), only enzymatic detergent (0.8%, 43°C, 5 min), only peroxyacetic acid (0.25%, 55°C, 12 min), only vaporized hydrogen peroxide gas (1.5 mg/l, 25°C, 3 h), or enzymatic detergent (0.8%, 43°C, 5 min) + vaporized hydrogen peroxide gas (1.5 mg/l, 25°C, 3 h) are insufficient for prion inactivation. SDS, sodium dodecyl sulfate.

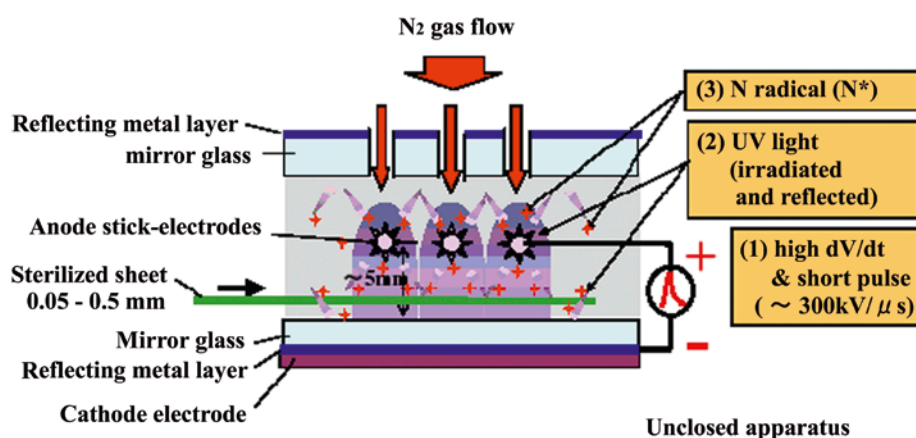


Figure 5. Constitution of  $N_2$  gas plasma sterilization apparatus. Atmosphere in the apparatus with an  $N_2$  gas flow. Under the conditions, high  $dV/dt$  and short pulses were applied, resulting in the generation of gas plasma. The possible sterilization mechanisms are as follows. (1) high  $dV/dt$  and short pulses, (2) UV exposure, (3) N radical. Cited from Fig. 1(a) in Shintani *et al.*, (40) with permission from Society for Antibacterial and Antifungal Agents, Japan. UV, ultraviolet.

nuclei and free electrons in a chamber containing active gases like vaporized hydrogen peroxide or inactive gases like nitrogen (40). The process is achieved by the supply of sufficient energy, in the form of heat or an electromagnetic field, to gas. Currently, there is only one commercial gas plasma sterilizer, Sterrad® (Johnson&Johnson, K.K.). Thus, most research on gas plasma sterilization is performed using this machine. Studies using gas plasma instruments including developing instruments beside those of prion (39) reported inactivation of human immunodeficiency virus (HIV) (41) hepatitis A virus (41), respiratory syncytial virus (RSV) (41), vaccinia virus (41), herpes simplex virus (HSV) (41), poliovirus (41), duck hepatitis B virus (42), bacterial spores (43-45) and endotoxin

(40). However, the sterilization mechanisms are not simple. In the case of Sterrad®, the mechanism of action seems to be the effect of vaporized hydrogen peroxide, not that of gas plasma. In this case, gas plasma appears to contribute to the elimination of remaining toxic vaporized hydrogen peroxide after the reaction. In contrast, nitrogen and noble gases (Argon and Helium) do not themselves have a biocidal effect, but their gas plasmas are effective for inactivation of pathogens. Therefore, gas plasmas of nitrogen and noble gases have complicated mechanisms of radical production, UV exposure and high  $dV/dt$  and short pulse (Fig. 5) (40). Such an approach using inactive gases is promising for the sterilization of fragile medical devices, especially endoscopes (46).



#### 4. Conclusions and perspectives

Prion has the unique feature of being devoid of nucleic acid. Therefore, its inactivation requires different procedures from those applied to other pathogens. In addition, prion diseases are zoonotic infectious diseases (5). Notably, the BSE prion causes vCJD in humans, and can be transmitted via blood (47). Current diagnostic methods for prion diseases are post-mortem, though recent research has achieved drastic improvements and enabled a highly sensitive diagnosis. Notably, PMCA raises the possibility of a pre-mortem diagnosis using blood samples not only from terminally ill individuals but also from preclinical onset (29). Gas plasma sterilization is a promising method potentially effective against all microorganisms including prions. This approach would offer profound advantages over conventional methods. By introducing appropriate gases into the system, certain gases critical for sterilizing each micro-organism may be elucidated.

Finally, the authors note that the information is based on scientific publications at the time of preparation. Therefore, the authors and publisher take no responsibility for any consequences of the application of any of the information in this review by any reader. Recently, guidelines for 'Disinfection and sterilization in healthcare facilities, 2008' by the Centers for Disease Control and Prevention (CDC) (48) and guidelines for control of prion diseases, 2008 by the Research Committee on Prion Disease and Slow Virus Infection, The Ministry of Health, Labour and Welfare of Japan (38) have been published. For clinical issues relating to disinfection and sterilization, these guidelines are appropriate for consultation.

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