Introduction

The residual risk of transfusion transmitted infection (TTI) had been decreased greatly since the donor screening methods, especially the nucleic acid testing (NAT), were introduced. NAT can shorten the period between infection and detection (window period) to several days after infection [1]. However, transfusion safety still faces challenges due to limitations of test sensitivity and more importantly the unexpected appearance of new pathogens. The newly discovered or re-emerging viruses such as West Nile virus, human parvovirus B19 and latest reported severe fever with thrombocytopenia syndrome bunya virus [2] in the donor blood endangered blood safety. Pathogen inactivation/ reduction (PI/PR) strategies provide last line of defence against various pathogens to secure transfusion safety.

PI/PR refers to any technology that inactivates or reduces all types of blood borne pathogens. Different methods were designed for different blood products such as plasma, platelets and red blood cells (RBCs). An ideal PI/PR is expected to inactivate all blood borne pathogens without damaging the quality of blood or blood products. Some common PI/PR methods include treatment with solvent/detergent (S/D), Methylene blue, Psoralens, Riboflavin. Some new technologies for PI/PR are under development.

PI/PR by Solvent/Detergent (S/D)

S/D technology is a combination of solvent and detergent. The most- commonly used protocol is: Treat blood sample with 1% tri-(N-butyl)-phosphate (TNBP) and 1% Triton X- 100 for 4 hours at 30°C, then remove S/D reagents by vegetable oil extraction and subsequent reverse-phase chromatography on C18 resin. This method inactivates pathogen by disrupting the lipid enveloped membrane, so it can’t be used for blood components with cellular structure. The S/D treatment was first licensed by the US FDA in 1985 for use in the manufacture of an anti-hemophilic factor (AHF) concentrate [3], and then applied in coagulation factors and pooled plasma. S/D can rapidly inactivate different lipid enveloped virus, such as vesicular stomatitis virus (VSV) (virus titer reduction ≥ 7.5log), sindbis virus (≥ 6.9log), HIV (≥ 6.2log), hepatitis B virus (HBV) (≥ 6log) and hepatitis C virus (HCV) (≥ 5log) [4]. However, S/D can’t inactivate non-enveloped virus, such as hepatitis A virus (HAV) and parvovirus B19. Various studies [5-7] showed that treatment of S/D could reduce the activity of coagulation factors (CFs), inhibitors, immunoglobulins and other plasma proteins by about 5-20%. S/D treatment is safe and the final S/D reagents removal step ensures the final product is non toxic.

PI/PR by Methylene Blue

Methylene blue (MB) is a phenothiazine compound that can be activated by visible light to generate reactive oxygen species (ROS), mostly singlet oxygen, through a Type II photodynamic reaction. These highly active molecules contribute to methylene blue’s pathogen-inactivating activity [8]. The first MB treatment system was developed by the Institute Springe in Germany. The conventional MB treatment included white blood cell (WBC)-reduction filtration, MB Pill dissolution, illumination and MB removal. Because of cell filtration step, this method was only applied to single donor plasma. The PI/PR efficacy of MB treatment for lipid enveloped virus is significant for both double and single- stranded RNA and DNA viruses, but for the non lipid-enveloped virus, the effect is inconsistent. Non-lipid enveloped virus like human parvovirus B19 could get a 4log or more reduction, while others like HAV are not affected by MB treatment [9]. Although MB can cross cell membrane by simple diffusion, its low concentration within cells makes it impossible to inactivate intracellular viruses, bacteria and protozoa. While it is effective for some pathogens, MB treatment affects the activity of some plasma proteins, such as CF VIII and fibrinogen [10,11]. MB PI/PR was licensed for use in Europe, Brazil, and Austria. The safety profile of MB PI/PR was validated by many studies, although allergic reactions to MB have been reported [12].

PI/PR by Psoralens

Psoralen is a naturally occurring photoactive substance found in a number of plants. PI/PR by psoralen has been successfully developed as a commercial product - INTERCEPT system [13]. It utilizes amotosalen, a synthetic psoralen (formerly S-59-HCl) as active compound. The amotosalen contains a tricyclic molecule structure, thus it can pass cellular membranes and interact with nuclei acids freely. Upon the illumination of UVA light (300-400 nm), it forms covalent cross links to pyrimidines in DNA and RNA and blocks the replication and transcription of mRNA. After treatment, the residual amotosalen and photoproducts are absorbed by silicon which is fixed in the treatment set. The psoralens PR were proved effective against almost all blood borne pathogens, including viruses, bacteria and protozoa. This method was approved by several Europe countries and applied for plasma and platelets. Studies show that this treatment slightly influences the function of blood components without damaging the overall quality [14]. The toxicological studies and clinical trials demonstrated that this treatment is safe [15].

PI/PR by Riboflavin

Riboflavin (vitamin B2) is present in food and natural products. Riboflavin combined with UVA (280-360 nm) was used to inactivate various pathogens. The UVA light can damage the nucleic acids of...
pathogens directly. In addition, the riboflavin can intercalate between bases of DNA and RNA. Upon light exposure, it leads to a Type 1 photochemical reaction and damages nucleic acids of pathogens irreversibly [16]. TERUMOBCT (a private company) developed a riboflavin PR system (Mirasol) for treatment of plasma and platelets [17]. The process of riboflavin PR is similar to other photochemical methods except no removal step for residual drug as the riboflavin is classified as a Generally Regarded as Safe (GARS) compound by the US FDA, and the toxicological assessment of its photoproducts show that they are free of safety concerns. Like the INTERCEPT system, it is effective against a wide range of enveloped and non-enveloped viruses, Gram-negative and Gram-positive bacteria, parasites, although its ability to inactivate HBV and HAV is weak according to the result of standard in vitro assays for infectivity (TCID50). The reduction activity for the two viruses is 2.5log and 1.6log, respectively. The quality of the Riboflavin-treated products shows no significant difference compared with non-treated products [18].

In addition to pathogen reduction methods discussed above, there are some other PR methods under development. Physical methods such as heat treatment and Nanofiltration were applied in plasma fractions [19]. Harald et al. reported a method using short-wave ultraviolet light (UVC) to reduce pathogen in platelet concentrates by its direct interaction with nucleic acids [20]. Under the illumination of 0.4 J/cm² UVC, no bacterial growth was observed 6 days after treatment. Bradley et al. investigated a pressure cycling technology derived from the industrial applications of food and vaccine PR to inactivate model pathogens in plasma [21]. With 2 minute cycles of 275 MPa or 345 MPa at -5°C, the phage titer of Lambda phage was reduced 4.2log or 6.9log, respectively. Since they only choose one pathogen to investigate, the method needs further evaluation on efficacy and safety.

In general, each PI/PR method has its advantages and disadvantages. Some methods have strong ability to inactivate specific pathogens but no broad spectrum, while others can inactivate a wide range of pathogens but the reduction activity is low. Up to now, PI/PR in plasma is well developed with many choices, but PI/PR methods for cellular components is still under development, particularly methods tailored for the red blood cells (RBCs) and whole blood. A chemical method using a compound (S-303) based on a derivative of quinine mustard was developed to inactivate pathogen in RBCs [22]. However, the development of antibody against neoantigens in transfused subjects stopped it in Phase III clinical trials. Another method using ethyleneimine (PEN110) to target RBCs PR [23] was also stopped in Phase III clinical trials with the same reason. Luckily, the INTERCEPT system has started Phase III clinical trials for RBCs and R. P. Goodrich et al investigated the Mirasol system for whole blood [24,25]. With the development of new technologies, PI/PR will provide last line of defense to ensure transfusion safety.

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