Current methods for the reduction of blood-borne pathogens: a comprehensive literature review

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Pathogen load of blood products

Current strategies to reduce the risk of transmissible, transfusion-associated infections rely on donor deferral and testing procedures, filtration or gamma irradiation of blood products to reduce the number of pathogens and viable donor leucocytes promoting adverse transfusion reactions in the recipient[1,2]. As a result of these strategies, morbidity and mortality from transfusion-transmitted infections, including parasites, are exceedingly rare[3]. In the developed Western world, risk estimates for traditional transfusion-associated viruses (hepatitis B and C viruses, human immunodeficiency virus) vary between 1×10^6 and $>10\times10^6$ [4,5]. Thus, the problem concerning these viruses seems to be managed. However, in non-developed countries not testing all blood products for viruses, the respective risk might be much greater. A residual risk still remains concerning pathogens for which no detection system currently exists (e.g. emerging pathogens) or which have a "window period", when blood levels of specific disease markers are too low for detection, e.g. shortly after infection. Nucleic acid testing has markedly reduced, but not completely eliminated this period. Meanwhile, bacterial contamination, especially of platelet concentrates (because of their storage at ambient temperature allowing for bacterial proliferation), is recognised as the most common cause of transfusion-transmitted infections. Up to 0.6% of platelet concentrates from routine production might be bacterially contaminated[6], and the estimated mortality risk from severe post-transfusion sepsis ranges from 1:230,000 to 1:625,000 donor exposures[7-9]. Thus, bacterial (not viral) contamination might become the driving force for broad implementation of pathogen-reduction technologies (PRT), which are able to inactivate bacteria, even anaerobic ones, below the detection limit of screening methods. Additionally, the latter require time for sufficient pathogen proliferation prior to detection (generally >36 hours)[10,11] and involve the risk of false-positive and false-negative test results[12].

Pathogen-reduction treatments involving solvent-detergent, β-propiolactone or nanofiltration have markedly increased the safety of plasma and its derivates but are not suitable for blood cells, as they irreversibly damage cellular membranes and function. PRT for cellular blood products are based on photosensitizers, which are added during processing and, after being activated by irradiation with visible or ultraviolet (UV) light, generate active oxygen species or use oxygen-independent electron transfer processes to damage nucleic acids (photodynamic reaction) or form irreversible covalent cross-links to prevent transcription, translation and growth (photochemical reaction). The rationale for targeting nucleic acids is that pathogens and white blood cells require nucleic acid function that is unnecessary for the therapeutic efficacy of platelets, plasma, and red blood cells. The reduction capacity should be at least 4-6 $\log_{10}$ steps for sufficient pathogen reduction.

Pathogen reduction technology methods for cellular blood products

Due to their capacity to inactivate donor leucocytes, PRT are considered as effective as gamma irradiation for the prevention of transfusion-associated graft-versus-host disease[13-15]. Studies on PRT-treated plasma or platelet concentrates are more advanced than those with red blood cells or whole blood. This may be because bacterial contamination is greatest in platelet concentrates. Moreover, red blood cells represent a more difficult environment because of the absorption spectrum of haemoglobin, its greater viscosity, and the prolonged storage time which increases, for example, haemolysis and potassium leakage. All PRT bear the potential to induce immune responses in the recipient, who can form antibodies that can bind to the altered blood cells and cause them to be cleared from the circulation.

Currently, the most intensive studied dyes with photodynamic properties are riboflavin, the essential vitamin B_2, and the phenothiazine derivative methylene blue, while dyes with photochemical properties include psoralens, such as S-59 (amotosalen-HCl) and S-303 or the ethylene imine PEN-110. The latter compounds also interfere with nucleic acids by alkylation chemistry but become activated by other mechanisms than an external light source, e.g. upon pH shift.

The INTERCEPT Blood System (Cerus Corporation, Concord, California, USA) is based on photochemical treatment using the psoralen amotosalen-HCl and UVA light[16-28], while the Mirasol PRT system (TerumoBCT Biotechnologies, Lakewood, Colorado, USA) and the

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THERAFLEX MB Plasma system (Blood Centre of the German Red Cross NSTOB, Institute Springe, in association with MacoPharma International GmbH, Tourcoing, France) use riboflavin or methylene blue in combination with UV or visible light, respectively (Table I). Until now, immune responses have not been reported for amotosalen-HCl, riboflavin or methylene blue, but have been seen for red blood cells treated with S-303 or PEN-110[29,30]. The THERAFLEX UV-Platelets system (Forschungsgemeinschaft der DRK Blutspendedienste [German Red Cross] in association with MacoPharma) uses UVC irradiation only, without the addition of any photosensitiser. Except for the INTERCEPT Blood System and the Mirasol PRT system, all systems for pathogen reduction of labile blood products are under intensive development but are not yet routinely available.

The INTERCEPT Blood System for platelet concentrates and plasma

This psoralen-based system is used in several European countries including Germany. A total of over 700,000 psoralen-UVA treated blood products have been transfused, and neither observational studies[24,27] nor the haemovigilance programme[28,31] have shown any unexpected safety concerns. The system has proven to have sufficient reduction capacity against many pathogens (up to 4-7 $\log_{10}$ steps) including bacteria. The efficiency with which non-enveloped viruses can be inactivated varies strongly. Of note, hepatitis A virus is not susceptible to inactivation[32].

Using this system, 17.5 mL of amotosalen-HCl (at a final concentration of 150 µM) is added to plasma or platelet concentrates resuspended in InterSol (Fenwal, Deerfied, Illinois, USA) or SSP+ (MacoPharma, Tourcoing, France). Thereafter, the mixture is illuminated with UVA light (320-400 nm, 4-6 min, dose 3 J/cm^2). Shorter wavelengths were shown to have detrimental effects on proteins through the generation of active oxygen species. After the photoreduction process, amotosalen and free photoproducts are adsorbed in a compound adsorption device for 10-20 minutes (plasma) or 4-16 hours (platelet concentrates). The treated blood component is then transferred into the final storage bag. As a result of adsorption in the compound adsorption device and several transfer steps, a volume and blood cell loss of about 12% may be observed[33].

The Mirasol PRT system for platelet concentrates, plasma and whole blood

This riboflavin-based system is currently under investigation in ongoing clinical trials (IPTAS, PRESS, PREPARES) and available for routine use in several places in Europe and the Middle East. The Mirasol PRT system has been shown to be effective against a variety of clinically relevant pathogens (reduction up to 4-6 $\log_{10}$ steps). It has demonstrated 98% efficacy against bacterial strains responsible for most of the severe infections following transfusion[25]. To date, it is the only PRT that has been demonstrated to inactivate the non-enveloped viruses such as hepatitis A virus, which is highly resistant to chemical and heat-mediated interventions[26]. Preliminary results in whole blood suggest good retention of blood cell function so that PRT of all blood products using the same system may become possible in the near future.

Riboflavin (35 mL at a final concentration of 50 µM) is added to the blood product, which is then irradiated with UV light (265-370 nm, for 4-6 minutes at a dose of 6.2 J/mL). Most of the applied energy is in the UVB range (280-315 nm), with a lesser amount in the UVA range (315-400 nm). The peak wavelength (313 nm) preferentially targets riboflavin-induced damage to nucleic acids and does not emit energies where cytochromes and other essential cofactors for mitochondrial function/activity absorb. As an essential vitamin, riboflavin and its photoproducts do not subsequently need to be removed from the treated blood component, thus minimising blood cell loss.

UVC irradiation of platelet concentrates and plasma

The THERAFLEX UV-Platelets system is currently under evaluation for its efficacy and safety. The process is based on the application of UVC light (200-280 nm) combined with intensive agitation. Since no photosensitiser needs to be added, toxicity-related adverse events associated with such agents can be excluded.

Table I - Most advanced methods for pathogen reduction of labile blood products.

	Photosensitiser + light source	Blood product	Kill power rate
INTERCEPT Blood System	amotosalen-HCl (psoralen) + UVA (320-400 nm)	FFP, PC	4-7 $\log_{10}$ steps
MIRASOL PRT system	riboflavin (vitamin B2) + (UA) UVB (265-370)	FFP, PC, RBC, WB	4-6 $\log_{10}$ steps
THERAFLEX UV-platelet system	None UVC irradiation (254 nm) only	PC	4-6 $\log_{10}$ steps
S-303 PRT system	S-303 (psoralen), glutathione activation upon pH shift	RBC, WB	4-6 $\log_{10}$ steps
TMB Plasma system	methylene blue + visible light (590 nm)	FFP	≥6 $\log_{10}$ steps

Legend
PRT: pathogen-reduction technology; UV: ultraviolet light; PLT: platelet; FFP: fresh frozen plasma; PC: platelet concentrate; RBC: packed red blood cells; WB: whole blood.

The irradiation process results predominantly in the formation of cyclobutane pyrimidine and pyrimidine-pyrimidine dimers blocking the elongation of nucleic acid transcripts. Given the different absorption characteristics of nucleic acids and proteins, the irradiation process mainly affects leucocytes and pathogens (by at least 4-6 $\log_{10}$ steps), while coagulation proteins and platelet function are largely preserved[34]. Platelet concentrates treated with this system after being spiked with high titres of cell culture-grown hepatitis C viruses were sufficiently inactivated, with a reduction factor of >5 $\log_{10}$[35]. The bovine viral diarrhoea virus (a model for hepatitis C virus) was, however, less sensitive to UVC irradiation. Furthermore, UVC irradiation failed to effectively inactivate spores (having a low impact on blood products), West Nile virus and, most importantly, human immunodeficiency virus HIV (for which screening is performed)[36,37]. Disruption of disulphide bonds of the fibrinogen receptor (glycoprotein IIb-IIIa), described for UVC irradiation as "platelet sunburn"[38] appears to be only slightly increased using the THERAFLEX UV procedure, as seen from the limited increase of free thiol groups on the platelet surface[34].

Resuspended platelets are transferred into a UVC permeable 19×38 cm irradiation bag. The UVC irradiation (254 nm, 20-30 s, dose 0.2 J/cm^2 [1 J/cm^2 for plasma]) is performed using a special UVC irradiation device (Macothronic, MacoPharma) with the bags placed loosely on a quartz plate. Since the pathogen-reduction capacity was shown to be greatest at ≥100 rotations per minute (rpm), the plate is agitated at 110 rpm. After irradiation, the platelets are transferred into their final storage container and are ready for transfusion without further processing.

The S-303 PRT system for red blood cells

This photochemical-based PRT system for erythrocytes has the capacity to reduce pathogens by 4-6 $\log_{10}$ steps[39]. S-303 is composed of an effector (acridine moiety), a linker (alkyl chain) and an anchor (mustard hydrochloride moiety). It is designed to target nucleic acids, cross-link them via a bis-alkylating group and release a negatively charged non-reactive by-product (S-300). S-300 is then captured by glutathione, also added to the blood component to minimise non-specific reactions with proteins. A second generation system was developed after the observation of an unexpected immune response in two of 16 patients suffering from chronic anaemia, who required more than a single transfusion of red blood cells for therapeutic support[29]. The technology has also shown promise for application to whole blood[40].

Thirty millilitres of glutathione and S-303 in saline are mixed with the blood product (to a final concentration of 200 mmoL/L glutathione and 0.2 mmoL/L S-303). The whole mixture is then transferred into a second container to allow both the pathogen reduction process (30 minutes) and the decomposition of S-303 to S-300 (6-18 hours). After centrifugation, the supernatant is removed, and the treated red blood cells are transferred into their final storage container containing additive solution for storage for up to 35 days at 4±2 °C.

The THERAFLEX MB (TMB) Plasma system for plasma

In contrast to PRT-treated platelet concentrates and red blood cells, PRT-treated plasma has been in clinical use for several years and proven effective in a variety of therapeutic settings[41,42]. More than four million methylene blue-treated plasma units, including about two million treated with the THERAFLEX system have been generated to date[43]. The pathogen killing power for most enveloped viruses reaches at least 6 $\log_{10}$ steps[44], but is considerably less for non-enveloped viruses. Plasma units treated with the TMB Plasma system after being spiked with high titres of hepatitis C virus were sufficiently inactivated below the detection limit already by $^1/_{12}$ of the full light dose, while BVDV was less sensitive[35]. The activities/life spans of clotting factors, including ADAMTS-13, are reduced by 10-35% in TMB-treated plasma[45]. Observational studies in Spain[46] and haemovigilance data from France[47], however, raise concern that methylene blue-treated plasma is probably less effective than quarantined plasma in the treatment of thrombotic thrombocytopenic purpura and may induce more severe, sometimes fatal allergic reactions.

At the start of use of the current TMB plasma system a 0.65 µm membrane filter (Plasmaflex PLAS4, MacoPharma) removes residual leucocytes, red blood cells, and platelets as well as microvesicles and microparticles. Thereafter, the filtered plasma flows past a dry "pill" containing 85 mg methylene blue ensuring a final concentration of 1 µM for a plasma volume ranging between 235 to 315 mL. The following illumination with visible light (590 nm, 20 min, dose 180 J/cm^2) is achieved by sodium low-pressure lamps or light emitting diodes in a special device (Macothronic, MacoPharma). After treatment, residual methylene blue and its photoproducts are removed by a special filter (Blueflex, MacoPharma) to an average level of 2 µg/L (0.5 µg per plasma unit).

Preclinical and clinical investigations

Pre-clinically, treatment of platelets with the INTERCEPT Blood System or the Mirasol PRT system was associated with increased acidity and cell activation, enhanced metabolism (glucose consumption, lactate production), and some impairment of *in-vitro* properties

(aggregation, extent of shape change, hypotonic shock response, etc.). The applied wavelength energy of the Mirasol PRT system is distinct from the absorbance energy of mitochondrial enzymes (370-450 nm). Unlike the psoralen-based technology, this allows maintenance of the oxidative phosphorylation pathway[48,49] considered crucial for platelet behaviour during clot formation at sites of vascular injury[50]. In the absence of mitochondrial respiration, reduced viability[51] and haemostatic effectiveness[52] may be undesirable side effects. The *in-vitro* quality of UVC-irradiated platelets was shown to be comparable to that of platelets treated with other PRT[17,18,53-55]. The heat shock response decreased by 20-30% immediately after UVC irradiation but recovered partly during storage[34].

Clinically, PRT-treated platelets produced lower post-transfusion corrected count increments, necessitating an average of 35% more transfusions[22,23,56]. This was mainly the result of the lower platelet dose after PRT treatment due to multiple bag transfers and use of compound adsorption devices, but development of an intrinsic storage lesion might also have contributed to this finding. As judged by the frequency of bleeding events, PRT-treated platelets were haemostatically as effective as their untreated counterparts; this was confirmed by a haemovigilance programme[28,31] and similar *in-vitro* aggregability under flow conditions[57]. However, a recent clinical investigation of psoralen-UVA-treated platelet concentrates was stopped prematurely because of significantly more haemorrhagic events[58]. In parallel, we observed that the shear-induced adhesion properties of psoralen-UVA-treated platelets was reduced (albeit not significantly so)[52]. A possible reason is that haemovigilance programmes may be too underpowered to really detect such discrepancies that might be overcome by increased transfusion doses. Given the equal frequencies of adverse transfusion reactions and refractory states, PRT-treated platelets were considered as safe as conventional platelet concentrates. Recovery and survival rates of radiolabelled PRT-treated platelets in healthy volunteers were reduced (even after UVC irradiation) but still acceptable for transfusion[59,60]. However, study sizes appear far too small to draw any firm conclusion in this respect.

Of the seven studies conducted with S-303-treated red blood cells, only one was performed using the second-generation process[39]. Former phase-III studies were suspended when two of 16 chronically transfused patients developed positive cross-match reactions to S-303-treated red blood cells. The underlying low-titre antibodies (that can also occur naturally[61]) were directed against the surface-bound acridine moiety of S-303[62]. A second-generation pathogen inactivation process was developed, minimising the amount of RBC-bound acridine. Preliminary results indicate that the treated red blood cells maintained sufficient viability (24-hour recovery rates of about 88%) and did not induce positive cross-matches in any sample[63].

Conclusion

Before broad implementation of PRT for labile blood products, it has to be shown that the photosensitisers and their photoproducts are extremely safe, robust in daily routine, and cost-effective, and that their efficacy can be controlled. Toxicological studies are difficult to perform and may not reveal rare events such as carcinogenicity, which can only be detected during long-term observation. Any benefit from the use of PRT-treated blood products may be offset by any incidence of an unanticipated adverse event[64]. Open questions still remain concerning the extent to which all pathogens, including unknown entities, non-enveloped viruses and prions, can be reduced. PRT-treated platelets seem to be functionally inferior to their untreated counterparts leading to lower corrected count increments and increased transfusion requirements in the first clinical trials. However, whether corrected count increments have the sensitivity and specificity to be really clinically relevant is a subject of debate[65]. Careful evaluation of bleeding events and platelet function before and after transfusion (e.g. via thrombelastography) appears more appropriate, highlighting the role of further clinical trials.

Keywords: pathogen-reduction technology, blood safety, UVC irradiation.

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