Investigation on the Development of Antimicrobial Polymer Brush Coatings for Platelet Storage Devices

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Introduction:

Microbial infection is one of the major complications associated with blood transfusion, especially platelet transfusion. According to Canadian Blood Services report, the rate of bacterial contamination of whole blood derived platelets is one per every 2000 units in spite of all the precautions taken during blood collection, processing, and storage. Considering the cost of antibacterial tests and the unwanted wastage of the stored platelets, it is very important to develop novel methods to prevent bacterial growth in stored platelets.

Currently platelets are stored in plasticized polyvinylchloride (pPVC) bags for transfusion. The hydrophobic surface of PVC bag is conducive of bacterial attachment and proliferation, and the biofilm formation. The hydrophobic surface of the PVC also induces platelet activation and platelet adhesion on to the surface of the bag. Thus development of a coating which can prevent both bacterial attachment (possibly antimicrobial), and highly compatible with platelets is an important first step towards a novel storage device for platelets.

Recently our lab has shown that biocompatible antimicrobial coatings based on surface attached antimicrobial peptides (AMPs) and polymer brushes can prevent biofilm formation and can kill adhered bacteria on the surface of a titanium implant. AMPs are components of innate immune system in vertebrates and invertebrates and show broad-spectrum activity against various bacteria.

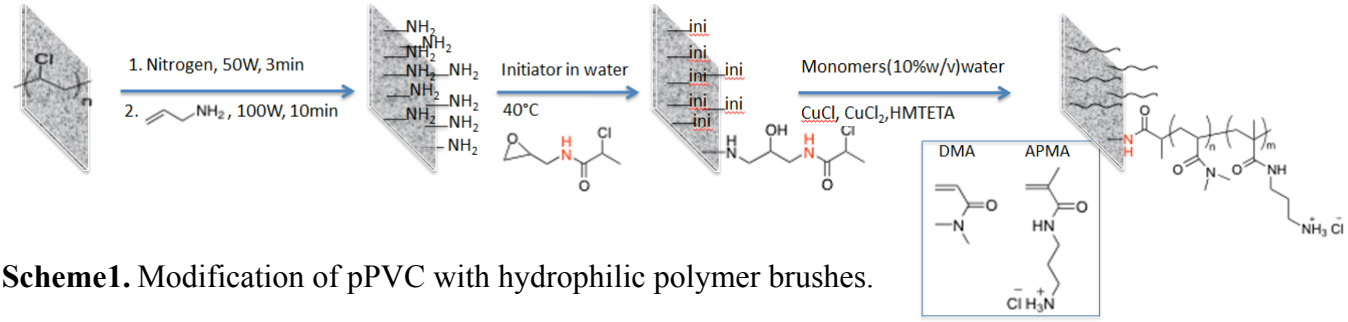
Based on these considerations our hypothesis is that modification of platelet bag surface with hydrophilic polymer brush and antimicrobial peptide based coating will enhance its biocompatibility and will make the surface antimicrobial. This dual functional coating will not only decrease the surface induced platelet damage but also prevent the bacterial growth.

Materials and Methods:

Hydrophilic polymer brushes were synthesized by Surface initiated Atom Transfer Radical Polymerization (Si-ATRP) of hydrophilic monomers on pPVC. Initially pPVC was functionalized with primary amine groups via allylamine plasma modification. Water-soluble ATRP initiators (2-chloro-*N*-(oxiran-2-ylmethyl)propanamide as an example) were reacted with amine groups on the surface and polymerization allowed to proceed in water at room temperature. Along with any desired hydrophilic monomer, an amine group containing monomer was also added to generate functional groups required for the next step (scheme 1). In the present case, *N,N*-dimethylacrylamide and *N*-(3-aminopropyl) methacrylamide hydrochloride (APMA) were used. To conjugate AMP to the polymer brushes, an amine to sulfhydryl crosslinker was used; the linker reacts with the amino group of polymer brush on one side and with sulfhydryl group of AMP on the other side. This functionalization of the polymer brush was investigated using dansyl cysteine, a sulfhydryl containing fluorophore.

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), water contact angle measurements and gel permeation chromatography (GPC) fitted with multi-angle laser light

scattering (MALLS) and refractive index (RI) detectors were used to characterize the surfaces. Currently the conjugation of antimicrobial peptides and platelet interaction studies are in progress.



Scheme1. Modification of pPVC with hydrophilic polymer brushes.

Results:

The growth of hydrophilic polymer brushes from medical grade plasticized PVC was confirmed by the ATR-FTIR, XPS, and static water contact angle measurements. PDMA (poly *N,N*-dimethylacrylamide)-modified PVC showed lower water contact angle (47±5°) compared to the pristine sample (102±4°). In ATR-FTIR spectrum of modified PVC, a strong amide absorption band at 1630 cm^{-1} confirmed the formation of PDMA brushes on pPVC surface. Using a cleavable initiator, the polymer chains cleaved from the surface gave the molecular weight (MW) around 120,000 Da.

To investigate the functionalization and possible tethering of AMPs to the polymer brushes, a sulfhydryl containing fluorescent probe, dansyl cysteine, was used. Dansyl cysteine was conjugated to the polymer brushes on PVC surface via two different cross linkers and fluorescence spectroscopy confirmed the success of procedure. Using a similar method for conjugating selected AMPs including Tet 20(KRWRIRVRVIRKC) and Tet 26(WIVVIWRRKRRRC) is in progress. In vitro biocompatibility tests and antimicrobial activity of the surfaces are to be completed.

The quality of the stored platelets will be assessed by platelets morphology analysis, measurements of platelet metabolism (pH, glucose, lactose, pO2, pCO2) and activation states of stored platelets by the expression CD62p. The growth of the externally inoculated *Staphylococcus aureus* and *Staphylococcus epidermidis* (most common sources of platelet contamination) are evaluated in presence of plasma and platelets and will be correlated with the properties of the coatings.

Discussion:

We aim to study the effect of molecular weight and surface concentration of polymer brushes and peptides on the quality of stored platelets. Further, controlling the properties of polymer brushes including their monomer composition, molecular weight, and surface density to produce the required number of functional groups to provide a range of surface concentration of AMPs on the surface are of interest. For these aims, a method to determine the graft density of polymer brushes and also surface concentration of AMPs must be developed. We anticipate that the proposed dual functional coating will prevent both platelet storage lesion associated with surface interaction as well as the bacterial growth in stored platelets. Since the coating is covalently conjugated to the surface of the bag, we predict leakage of either polymer or peptides to the stored platelets will not occur and the coating can introduce a new pathway for developing safe blood transfusion devices.

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