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Extended Materials and Methods

Bacterial Strains

Although routine testing of red blood cells (RBCs) has not been implemented by blood manufacturers, an in-house quality control sterility program requiring bacterial testing with the BacT/ALERT system of expired blood components, including RBCs, was put in place at Canadian Blood Services in 2009 [1]. Analysis of the data obtained from this program, showed that although *P. acnes* was isolated from PCs, unexpectedly, *P. acnes* was the microorganism most frequently isolated from RBCs. 36,324 RBC units were tested from November 2009 to October 2013 with 11 true positive cultures (0.03%, classified according to AABB Bulletin #04-07). Ten of these 11 cultures (90.1%) were identified as *P. acnes*. It was therefore decided to include *P. acnes* strains isolated from both RBCs and PCs during quality control sterility testing in the present study. Nine *P. acnes* comprising five strains isolated from RBCs (CBS 11001, CBS 11026, CBS 11027, CBS 11031, CBS 11033) and four strains isolated from PCs (CBS11002, CBS 11028, CBS 11032, CBS 11034) at Canadian Blood Services in addition to four *S. saccharolyticus* strains (CNS1025548, CNS1025549, CNS1025550, CNS1025673) isolated during routine platelet screening at the Sanquin Blood Supply Foundation [2] were included in this study. *P. acnes* ATCC 6919, purchased from the American Type Culture Collection (ATCC, Manassas, VA), was used as a biofilm-positive control strain.

Platelet Concentrates

Buffy coat platelet pools used in this study were prepared at the Canadian Blood Services Network Centre for Applied Development (netCAD, Vancouver, BC) according to Canadian
Blood Services standard operating procedures. The use of these platelet pools for research purposes was approved by the Canadian Blood Services Research Ethics Board.

**Biofilm Assays**

The biofilm forming abilities of all the listed strains were tested in PCs and media for comparison. Bacterial cultures were adjusted to 0.1 OD$_{600}$ [$\sim 10^8$ Colony Forming Units (CFU)/ml for *P. acnes*, and $\sim 10^7$ CFU/ml for *S. Saccharolyticus*] in media or in PCs. Three ml of each bacterial suspension were added in duplicate to the wells of a 12-well flat bottom cell culture plate (Linbro, MP Biomedicals, LLC, Ohio) containing media or PCs. Media cultures [brain heart infusion (BHI) broth (BD Biosciences, Franklin Lakes, NJ) for *P. acnes* or trypticase soy broth (TSB, BD Biosciences, Franklin Lakes, NJ) for *S. saccharolyticus*] were supplemented with 0.6% glucose as this is requirement for bacterial biofilm formation [3]. Cultures in media were incubated at 37$^\circ$C under anaerobic, static conditions for 72 h while PC cultures were incubated under standard platelet storage conditions for five days. Following the incubation period, the planktonic (free-floating) cells were removed from each well. The wells were then gently washed three times with PBS, air dried and heat-fixed at 65$^\circ$C for 5 minutes. Three ml of crystal violet (CV) were added to each well and incubated at room temperature for 30 min; the excess stain was removed and the wells were washed three times with PBS followed by distaining for 15 min with a mix of 80% ethanol and 20% acetone. 180 ul of the distained solution was then added to the wells of a 96-well flat bottom micro titre plate and read at 492 nm. Wells containing media or PCs alone (negative control) were used to determine the associated background. All biofilm assays were performed three times, and each assay contained duplicate test wells.
Adherence to the plastic of platelet containers and scanning electron microscopy (SEM)

Two different approaches were taken to determine whether *P. acnes* and *S. saccharolyticus* could adhere to the plastic of platelet unit bags. Biofilm positive strains *P. acnes* CBS11031 and *S. saccharolyticus* CNS1025549 were used in these experiments. Since we have shown that biofilm negative strains of other coagulase negative staphylococci display a biofilm positive phenotype in PCs [3], we also analyzed adherence to the plastic of platelet containers by the biofilm negative strain *S. saccharolyticus* CNS1025673. In one approach, 1cm$^2$ coupons were aseptically cut out of unused sterile platelet concentrate storage units. The coupons were then adhered to the bottom of the wells of 12-well culture plates followed inoculation with *P. acnes* or *S. saccharolyticus* to concentrations of approximately $10^8$ CFU/ml and $10^7$ CFU/ml, respectively, and incubation under platelet storage conditions. After the incubation period, planktonic cells were removed manually from each well, the wells were then washed three times with PBS and then the coupons were gently lifted out of the well and placed in a sterile petri dish until further processing. In the second approach, PCs were aseptically removed from platelet units retaining a volume of approximately 60 ml within the unit. Bags were then inoculated to a final concentration of approximately $10^8$ CFU/ml of *P. acnes* or $10^7$ CFU/ml of *S. saccharolyticus* and incubated under platelet storage conditions. Following incubation, the PC units were emptied and the bags were washed twice with PBS. After removal of remaining PBS, coupons measuring 1cm$^2$ were aseptically cut out of the bags and placed in sterile petri dishes. The coupons obtained by either method were then fixed in 0.1 M cacodylate (Na(CH3)$_2$AsO$_4$$\cdot$3H$_2$O (Sigma-Aldrich, Inc., St. Louis, MO, USA) containing 2.5% glutaraldehyde (J.B. EM Services, Inc., Dorval, QC) for 2 h at room temperature, followed by dehydration with an ethanol gradient (20%, 40%, 60%,...
80%, 95%, 100%) and critical-point drying. The coupons were sputtered with a 20 nm layer of gold particles and visualized by using the XL30 ESEM microscope (Philips, Eindhoven, Netherlands).

References

