Microgel-based materials for augmentation of hemostasis in neonatal cardiac surgery patients

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Statement of Purpose: Bleeding after cardiopulmonary bypass (CPB) is a serious complication of cardiac surgery and is associated with substantial morbidity and mortality. Neonates in particular undergo long, complex congenital cardiac repairs with extensive suture lines and are vulnerable to post-CPB bleeding. In these patients, transfusion post-CPB is a necessity. Transfused blood products consist primarily of platelets and fibrinogen (in the form of cryoprecipitate) prepared from adult blood. Although the transfusion of adult blood products to pediatric patients is performed routinely to control blood loss, reduction in the usage of transfused blood products has been shown to correlate with better patient outcomes. Unfortunately, few alternatives exist, highlighting the need for better methods to control bleeding. Hemostasis involves the formation of a platelet plug embedded within a fibrin mesh. However, clot formation is impaired in neonates (less than 30 days of age). Coagulation factors do not cross the placental barrier, and neonates are known to have deficiencies in several coagulation factors at birth including factors II, VII, IX, and X. Furthermore, recent studies in our lab demonstrate that optimal polymerization conditions (pH/ionic strength) for neonatal fibrinogen differ from that of adult fibrinogen. Neonatal platelets are also known to be hyporeactive. CPB further impairs these already compromised platelets and dilutes clotting factors. In this project we explore the application of microgel-based materials to augment clotting in neonatal CPB patients. First, we utilize microgel based platelet-like particles (PLPs) that recapitulate key hemostatic functions of platelets to augment hemostasis in patient plasma samples. We then utilize solvent swollen, microgel films to modulate the pH and ionic strength of plasma samples to enhance clotting.

Methods: After IRB approval, 16 neonates scheduled for elective cardiac surgery and CPB at Children's Healthcare of Atlanta were enrolled. Samples were collected at baseline, post-CPB and post transfusion. PLPs were produced as previously described[1] by conjugating humanized synthetic single domain variable fragment (sdFv) antibodies with high affinity for fibrin to highly deformable, ultra-low crosslinked poly(*N*-isopropylacrylamide) (pNIPAm) hydrogel microparticles (μgels) using EDC/NHS coupling. To characterize the effect of PLPs on clotting *in vitro*, we utilized an endothelialized microfluidics device that accurately recapitulates the cellular, physical, and hemodynamic environment of microcirculation.[2] Clotting of post-CBP platelet poor plasma (PPP) in the absence or presence of PLPs was analyzed in real time using microscopy. We then characterized the effect of PLPs on fibrin network structure using confocal microscopy and compared to both baseline and post-transfusion samples. For microgel film studies, microgels composed of pNIPAm, acrylic acid, and crosslinked with *N,N'*-methylenebisacrylamide were combined with the polycation, polyethyleneimine (pEI) and centrifuged to produce a thick microgel film. Films were hydrated with 25 mM HEPES buffer with a pH of 5, 7.4 or 9 and NaCl concentrations of 25 mM, 150 mM or 250 mM. Clotting of adult and baseline PPP was analyzed by monitoring clotting of plasma in contact with the films through gross clotting assays and real time confocal microscopy.

Results: *In vitro* clotting experiments in an endothelialized microfluidic device demonstrate robust clotting of post-CBP PPP in the presence of PLPs (Figure 1A-B) while minimal clotting is observed in post-CPB PPP alone. Confocal microscopy analyzing the effect of PLPs on fibrin network formation demonstrated that PLPs result in a denser fibrin network compared to post-CBP PPP alone (Figure 1C-D). Additionally, microgel films were found to augment clot formation under conditions of higher ionic strength and medium to high pH.

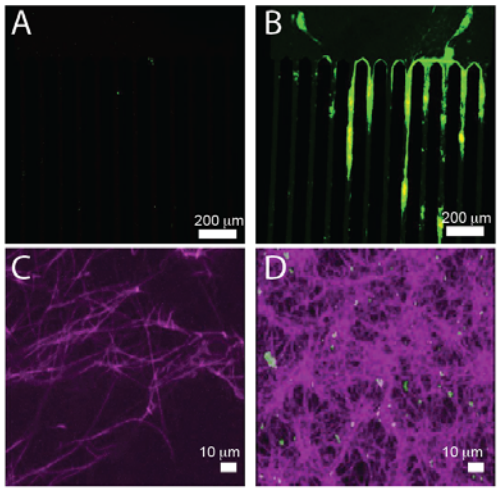

Figure 1. *In vitro* clotting assays with neonatal platelet poor plasma (PPP) collected after cardiopulmonary bypass (CBP). Clotting was assayed in an endothelialized microfluidics assay in the absence (A) or presence of PLPs (B). Fibrin=green; PLPs=red. The structure of post-CBP plasma was analyzed in the absence (C) or presence of PLPs (D) using confocal microscopy. Fibrin=magenta; PLPs=green.

Conclusions: Microgel based biomaterials, both PLPs and thick films, enhance fibrin clot formation *in vitro*. Such materials could greatly improve treatment of postsurgical bleeding in coagulopathic neonatal patients.

References:

1. Brown, AC, *et al*. Nat Mat. 2014.
2. Myers, DR, *et al*. J Vis Exp. 2012.