Hemocompatibility evaluation of elastomeric hollow fiber membranes as vascular grafts

Ángel E. Mercado-Pagán, Dai Fei Elmer Ker, Yunzhi Yang
Department of Orthopedic Surgery, Stanford University School of Medicine

Statement of Purpose: One of the main challenges for translation of small diameter vascular grafts (SDVG) into the clinical environment is their limited hemocompatibility. Material selection for vascular grafts should be done as to prevent long-term potential risks of restenosis, fouling, and thrombus formation [1]. In our lab, we have developed elastomeric hollow fiber membranes (HFM) as candidates for SDVG [2]. Here, we present our results for *in vitro* hemocompatibility testing of our HFM.

Methods: Polyester urethane HFMs were prepared by using a spinneret with concentric tubular outlets by a phase inversion method [3]. Water was perfused through the inner tube, while a polymer solution was pumped through the outer cylinder. The annular outflow was then collected in a coagulant bath as solid HFMs. Fresh blood was collected from a healthy donor and used for hemocompatibility testing. Two different setups were selected: perfusion and static. For the perfusion setup, the HFMs were connected on one end to a syringe with blood, and on another end to a collecting vessel (Figure 1). Blood was then pumped through the HFMs at a low rate. For the static experimental setup, the HFMs were directly immersed in blood. Extent of hemolysis and hemoglobin (Hb) release was determined by using a cyanmethemoglobin (CMH) assay after 2 hours of exposure to blood. A lactate dehydrogenase (LDH) assay was used to determine the total viability of cells in blood in contact with the HFMs, using the same setups. For protein adhesion, solutions of lysozyme, bovine serum album (BSA), human serum albumin (HSA), and fibrinogen were prepared. HFM samples were contacted with all the solutions for 1, 2, 3, 12, and 24 hours. The amount of protein attached to the HFMs was obtained by washing away the collected samples with a 1% sodium dodecyl sulfate (SDS) solution, and measured by using a micro-bicinchoninic acid (BCA) assay. For platelet adhesion, fresh human platelet rich plasma (PRP) was obtained, and samples were placed directly on dilute PRP solutions (10^6 platelets/mL) for 1, 2, 3, 12, and 24 hours. After each time point, samples were collected, washed, and lysed with platelet lysis buffer. The amount of platelets was related to the amount of LDH activity. Commercially-available medical-grade polymer segments or glass were used as controls for all experiments.

Figure 1. Blood perfusion setup for HFM. Arrow indicates HFM placed between collection chambers.

Results: CMH assay results indicated no significant difference in Hb concentration between untreated blood and blood in contact with HFMs, or between medical polyester materials and the HFMs, for both perfusion and static setups. Glass and pure water were used as positive controls, and as expected, the damage was significantly higher than in all other groups. SEM imaging of fixed HFMs from the perfusion setup showed very low erythrocyte damage. Figure 2A shows cell integrity on representative HFM as measured by the LDH assay under static and perfusion setups. Triton X100 refers to samples intentionally lysed with detergent for maximum damage. No significant differences were observed between perfused blood and untreated blood, or between HFM groups and medical-grade polylactic acid (PLLA). Representative profiles for protein adhesion are shown in Figure 2B. No significant differences were observed between the HFM and the polytetrafluouroethylene (PTFE) control group. Adhesion profiles seemed to stabilize after 12 hours, with maximum absorptions of 80-120 μg/cm^2 for all proteins. Figure 2C shows characteristic platelet adhesion profiles for HFM samples under static culture. Glass was used for maximum platelet adhesion. Platelet adhesion was comparable for all HFM to medical-grade PTFE.

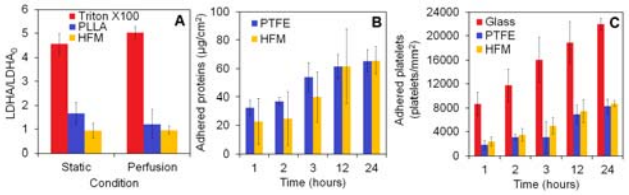

Figure 2. Representative results for HFM hemocompatibility testing: (A) LDH assay (B) protein adhesion (C) platelet adhesion

Conclusions: Our results showed excellent *in vitro* compatibility to human blood. The polymer HFMs do not damage the integrity of erythrocytes or other cells, which would greatly reduce function and activity of blood, and show low protein and platelet adhesion, which could lead to thrombosis after implantation. However, *in vitro* behavior could significantly differ from that *in vivo*. As such, the next step involves the implantation of our HFM grafts into animal models to test for graft viability. We also expect to modify our HFM with biologically-based vessel components, such as elastin, to improve integration *in vivo*. Optimization of mechanically-resilient and hemocompatible elastomer HFM vessel grafts could lead to improved vascularization of engineered scaffolds for organ repair.

References

[1] Thomas LV. Int J Cardiol. 2013;167:1091-1100.
[2] Mercado, A. Society for Biomaterials. 2014.
[3] Wen X. Biomaterials. 2006;27:3800-3809.

Acknowdgements: NIH R01AR057837 (NIAMS), NIH R01DE021468 (NIDCR), DOD W81XWH-10-1-0966 (PRORP).