Validation of a Novel Flow Circuit Assessing Vascular Biomaterials Retention of Endothelial Cells

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Statement of Purpose: We report here the validation of a novel optical/rheometrical platform with a flow system capable of *in vitro* simulating the pulsatile-flow waveform and fluid viscosity of physiological arterial and vascular circulation. We determined the *in vitro* conditions representative of the physiological *in vivo* environment through the endothelial cell retention on vascular grafts.

Methods: The rheometer (Anton-Parr, RheolabQC) shown in Fig. 1, based on concentric cylinders induces sheer in the fluid filling narrow gap between the rotating spindle and the outer glass cylinder. The outer jacket insures a controlled temperature and allows visual inspection of the biomaterial. The samples are supported on the middle spindle.

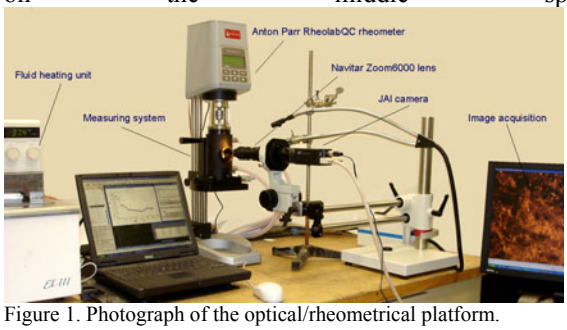

Figure 1. Photograph of the optical/rheometrical platform.

The sheer fluid designed to mimic the viscoelastic properties of whole blood while retaining typical cell medium properties is based on: DMEM (Sigma Aldrich, Canada), added with 10% FBS (Gibbco, Canada), and 0.1wt% Xanthan gum (Sigma Aldrich, Canada). The blood analog was sterilization by 254-nm ultraviolet (UV) irradiation for 3 hrs before its toxicity was assayed via the MTT assay. Briefly, $2.0x10^5/cm^2$ Human Aortic Endothelial Cells (HAoEC) (PromoCells, Germany) were inoculated in 1 ml of the solution for up to 14 days and assayed for cell viability.

Dacron vascular scaffolds (Boston Scientific Medi-Tech, Wayne, NJ USA), seeded with $2.0x10^5/cm^2$ HAoEC were statically co-incubated for 3 days before transfer to the vascular rheometer. The following physiologically reflective conditions were investigated on HAoEC attachment on Dacron vascular grafts: a) arterial shear stress 300 s^{-1} versus venous shear stress 100 s^{-1}; b) pulsatille: alternating shear rates of 300 s^{-1} (during 0.4 s) and 10 s^{-1} (during 0.6 s), cycle repeated 500 times versus continuous flow at 300 s^{-1} for 30 min; and c) a 30 min step-wise increasing conditioning step. The effects of the various rheological conditions on cellular retention were assessed by 2X washing the constructs and cell number counting by: a) cells fixation through a series of alcohol dehydration steps and visualizing the cells- vascular scaffolds through Scanning electron microscopy (SEM); b) assaying the cellular viability by the Alamar Blue™ (Invitrogen, Canada) viability assay.

Results: The MTT assay revealed constant cell viability values of at least 85% for 2 wks of HAoEC when grown in the blood analog medium. The grafts-cellular co-cultures submitted under the rheometer system reveled the following results: under continuous sheer stress at $300s^{-1}$ less than 10% of the cells remained on the vascular graft after a 60 minute-period. However, under a pulsatile flow alternating, at least 30% of the cells remained attached to the vascular graft after a 60 minute-period. The 3X higher cellular retention under a pulsatille flow was expected as the pulsatille flow better mimics the physiological flow. The addition of a 30 min pre conditioning step induced a 50% increase in cellular retention. Furthermore, there was not a significant difference between the arterial shear stress and the venous shear stress in the cellular retention (retention rate ~45%) when both conditions were applied in a pulsatille manner with a 30 min step-wise increasing pre conditioning step.

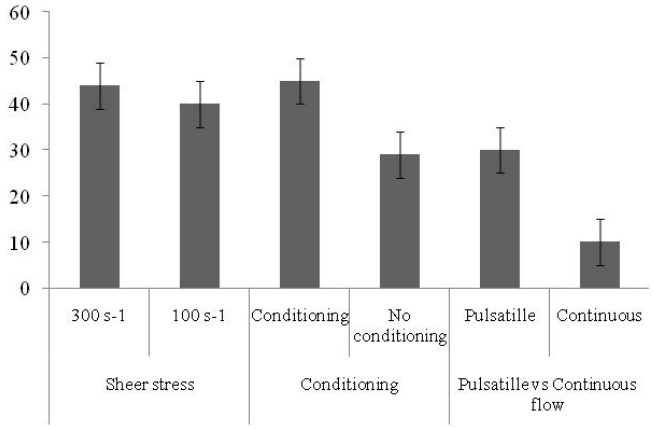

Figure 2. Cellular number remaining (in %) on vascular grafts following various rheological conditions as counted from SEM photomicrograph

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Conclusions: We have validated a novel an *in vitro* flow system that successfully simulates the physiological arterial and vascular flow. In addition this system is simple to manipulate and adapts well to vascular grafts-cellular co-cultures. Additionally the hemodynamic variables of shear stress are easy to induce obtaining the desired physiological conditions. Lastly this rheometer also incorporates a fluid that mimics whole blood viscoelastic properties (same shear stress-shear rate curves) and supports long term cellular growth. This method provides quick and efficient results for screening tests related to vascular graft selection and development.