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Performance Goal Text:	
Task Description:	The recent report of complete and partial thrombosis of the internal jugular vein in crew members on the International Space Station (ISS) raises concerns for the potential of life threatening pulmonary emboli. There appear to be very abnormal blood flow patterns in the internal jugular vein in zero gravity that may predispose to local thrombus formation. Given that the endothelium is uniquely sensitive to changes in the local flow/shear stress environment, the focus of this proposal is on the potential role of the endothelium in mediating localized thrombus formation. The purpose of this proposal is to identify changes in gene expression in venous endothelium exposed to the same flow patterns as those observed in the ISS crew members using isolated vein organ culture system. These studies will help us to develop a better understanding of the basic mechanisms responsible for thrombosis formation with the ultimate goal of potentially identifying biomarkers that would enable screening and risk stratification of crew members.
Rationale for HRP Directed Research	
Research Impact/Earth Benefits:	This work will help us to better understand the fundamental physiologic impact of altered flow patterns on the venous endothelium. In addition to allowing us to better understand the differences between venous and arterial endothelial responses, the work may have direct applications to helping us to understand the pathophysiology of venous disease. Finally, venous grafts are used in peripheral and coronary artery bypass surgery and we know that they have a limited, useful lifespan. Given that these segments are placed in a flow environment that is different from the natural flow environment, the work in this proposal may provide insight into how venous endothelium responds to non-physiologic flow stimuli.
Task Progress:	The major focus over the past year has been trying to increase the viability and number of the cells that we are collecting from the veins. Unlike our experience with arterial tissue, this has been a very significant technical challenge. In terms of collecting cells from the rodent inferior vena cava, we first attempted an endothelial-specific isolation using a variety of collagenase-based cocktails. We were able to develop a relatively pure collection of endothelial cells (ECs), but these cell numbers were insufficient for quantitative single-cell analysis. We then moved on to whole-tissue digestion and attempted to evaluate a series of different digestion cocktails that would preserve the viability of the cells that we were collecting. We ultimately used a cocktail that contained warmed collagenase/dispase [0.2%/0.2%] which resulted in increased cell numbers but an insufficient number of endothelial cells. This is due to the relatively low ratio of endothelial cells to total cells in the tissue. We retired the collagenase/dispase [0.2%/0.2%] perfusion approach which again gave us issues with cell viability.
	We then consulted with another lab that has been using a different technique on the mouse carotid artery for endothelial cell harvest. They found that flushing the internal carotid with QAIzol lysis reagent resulted in the isolation of relatively pure mRNA from endothelial cells. We found that when we used this approach, we did get good quality mRNA. Before undertaking sequencing, we performed standard PCR comparing the isolated material from the flushing approach with the mRNA isolated from digesting the entire vessel. We specifically targeted smooth muscle and endothelial markers (alpha smooth muscle actin and CD-31/PECAM) to quantify purity. We were very disappointed to find that in contrast to republished work, we found that this technique resulted in a very high level of contamination by vascular smooth muscle cells and relatively low expression of endothelial cell markers.
	Our current strategy is to focus on the human tissues with larger amounts of cells and we have applied a new digestion protocol using a cocktail of Liberase <sup>TM</sup> , elastase, and DNAse to digest the entire vessel segment. To overcome the low number of ECs isolated for scRNAseq, we will use fluorescent-activated cell sorting (FACS) to increase the proportion of ECs submitted for scRNAseq. CD31 will be used to sort the cells and the final mix of cells will include 50% CD31 positive cells and 50% CD31 negative cells. This will yield an endothelial cell-enriched sample for single-cell RNA sequencing.
Bibliography Type:	Description: (Last Updated: )