ORGAN ON CHIP PROPOSAL

BME395 – Group 3

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BODY ON A CHIP PROPOSAL

IMMUNE AND CARDIOVASCULAR SYSTEMS

A - EXECUTIVE SUMMARY

This proposal is a response to DARPA's RFA, which seeks to develop an in vitro platform that mimics human physiological systems for drug testing. [1] The objective of this proposal is to design an immune and cardiovascular system on a chip. The organ of choice for modeling the immune system on a chip is the bone marrow, which is the primary site for blood cells formation, antigen processing, antibody synthesis, and removal of senescent and abnormal cells. [2] The immune system chip contains a novel design of simulated bone marrow using blood stem cells embedded inside bone tissue-cultured peptide-hydrogel matrix, by combining the works of Torisawa [3] and Sharma [4]. In addition to the drug validation, the system will be verified for blood cell proliferation and differentiation through microfluidic blood counts. The key challenge is that the construct has never been tested and will require multiple iterations to optimize the system. For the cardiovascular system, a 3D co-culture microfluidic system of arteriole is presented, consisting of a circular cross-section lumen, a basement membrane of the endothelium, a collagen gel matrix and a perfusion system as a supplier of media and appropriate shear stress. System validation incorporates testing morphological structure, permeability, biocompatibility, and response to drugs. A key challenge of the arteriole model is the complexity of the fabrication protocol and thus flexible injection molding is beneficial. Design of a 3D in vitro model of atherosclerotic arterioles is presented, which is achieved by simulating hypertension (to induce endothelial dysfunction), and hyperlipidemia (to initiate plaque buildup). Drug testing validation will use computational methods for pharmacokinetics and flow analysis.

B – DETAILED TECHNICAL APPROACH

IMMUNE SYSTEM

Specific Aim: Emulate the physiology of the hematopoietic stem cell (HSC) differential response to drugs in vitro.

Hypothesis: The simulated bone marrow microenvironment enables the HSCs to proliferate and differentiate into the different cell lines, similar to natural physiological conditions.

Experimental plan: The chip will be made of poly-dimethylsiloxane (PDMS), with a microfluidic channel running to the bone marrow tissue culture chamber, and an exit channel into the microfluidic complete blood count (CBC) apparatus for validation of blood differential test (see Figure 1). [3] The tissue culture chamber will be a detachable cylindrical structure made of PDMS that is sandwiched between two layers of PDMS plates. [3] Prior to the drug testing protocol, the tissue will be cultured in a 3D co-culture system. Mesenchymal stromal cells will be seeded on a pre-set, medium-equilibrated 0.5% BD

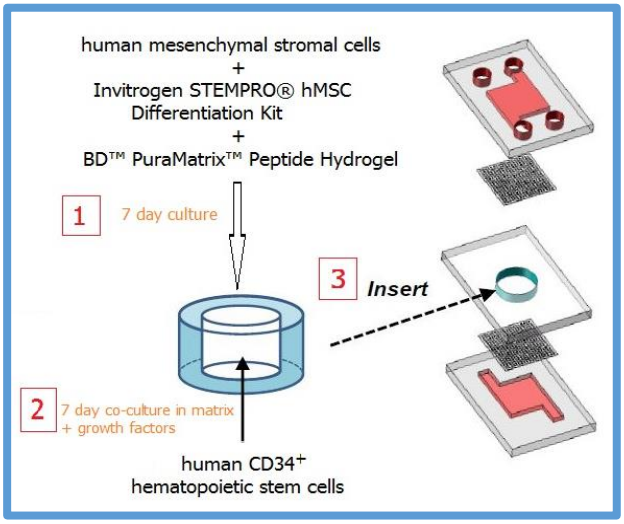

Figure 1: Diagram of the planned culture chamber and microfluidics apparatus. The bone marrow tissue will be within the detachable cylindrical structure, in blue.

PuraMatrix Peptide Hydrogel [5] inside the removable culture chamber. The matrix will be cultured with adipogenic, osteogenic and chondrogenic differentiation media [6] and will be verified for adipose, cartilage and bone formation using oil red O, alizarin red and alcian blue. [4] This marrow-mimicking microenvironment will create a hypoxia-gradient that is important for HSC differentiation. Freshly isolated human $CD34^+$ HSCs will be seeded in 7-day old 3D-cultures. The cultures will be fed every third day with growth medium supplemented with human specific growth factors (Stem Cell Factor-(SCF); Interleukin (IL)-6 and IL-3; concentrations as described by Sharma, et. al. [4]) then harvested after 7 days of co-culture. The chamber will then be inserted into the chip. [4] Sustained differentiation of the HSCs involves multiple growth factors and cytokines that are secreted by certain leukocytes and by tissues from other organs. These include erythropoietin, burst promoting activity (BPA) factor, interleukins, colony stimulating factors (M-CSF, GM-CSF, and G-CSF), thrombopoietin and tumor necrosis factor (TNF). [7] To compensate the lack of several growth factors, they will be perfused into the culture chamber through peripheral microchannels at specified time intervals during culture and drug testing.

System Validation: Blood tests and drug validations will ensure that the immune system on a chip mimics that of an in vivo system. A CBC will be carried to test the marrow's ability to differentiate HSCs into red blood cells (RBCs) and white blood cells (WBCs). Standard values for a CBC for an adult human are: an RBC count of 3.9-5.72 trillion cells/L and WBC count of 3.5-10.5 billion cells/L. [8] Additionally, a blood differential test will measure the percentage of each type of WBCs produced in by the marrow. Normal results for the blood differential tests are: neutrophils: 40% to 60%; lymphocytes: 20% to 40%; monocytes: 2% to 8%; eosinophils: 1% to 4%; other: 0.5% to 4%. [9] For phase one of the project, the blood tests will be performed off-chip. Phase two includes integrating the cell count mechanisms directly onto our system chip following Tai et al.'s [10] method for leukocyte differential count.

Drug Test: The drug tests will measure the effectiveness of the bone marrow system to proliferate and differentiate into the different expected cell types. The drugs chosen are in the active form, and as such do not require the liver or other organs for activation. The first drug is Mercaptopurine, which is converted into thioinosinic acid and 6-methylthioinosinate and inhibits glutamine-5-phosphoribosylpyrophosphate amidotransferase, preventing purine ribonucleotide synthesis [11]. This drug would prevent blood cell proliferation, resulting in low red and white blood cell count. While effective, it is a known carcinogenic [12], thus unsafe for this and other connected systems. The second drug is erythropoietin (introduced at significantly higher levels than perfused), which stimulates red blood cell production. If the drug affects the system, the CBC should show an increase in red blood cell count [13]. Computational models will be used for pharmacokinetics analysis.

DESIGN OF AN ARTERIOLE ON A CHIP

Specific Aim: Model a functional arteriole with a 3D co-culture system of Human Umbilical Vein Endothelial Cells (HUVECs) and Human umbilical cord arterial smooth muscle cells (HUASMCs) along with appropriate shear stress

Hypothesis: HUASMCs and HUVECs co-culture enable cell signaling between endothelium and smooth muscle layer and thus allow the smooth muscle layer to respond to the effects of intravenous drugs.

Technical Rationale: Blood vessels vary in diameter and composition in different parts of the circulatory system. The inner lining of blood vessels consists of a monolayer of endothelial cells

(ECs) situated underneath a basement membrane, which is surrounded by a dense layer of smooth muscle cells. The spatial organization of cells is integral because it determines cell response to cues from extracellular matrix, mechanical strength and tissue organization [14]. ECs *in vivo* are under hemodynamic shear stress and these forces have been shown to contribute to endothelial morphology, endothelial permeability, vasoregulation, and arterial remodeling [15]. Blood-flow patterns and resulting shear stresses on the vascular walls need to be selected aptly, as they vary along the vascular system [16]. A lumen with a circular cross-section may improve our *in vitro* approach as it has been shown that leukocytes migrate towards the corners of a rectangular cross-section channel [16].

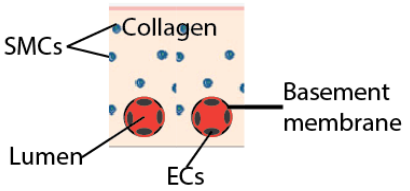

Figure 2 Cross section of lumen and collagen matrix

Experimental plan: A 3D microfluidic model of an arteriole that incorporates a circular cross-section of a lumen, spatial organization of cell types, and flow-induced shear stress is presented (see Figure 2). HUVECs will be used to create an endothelium. The endothelium will be coated with adhesion proteins, including human fibronectin and laminin, mimicking a basement membrane. HUASMCs will be seeded inside the pre-polymerized collagen matrix to constitute the smooth muscle layer. The collagen gel will be made with type I collagen (rat tails), diluted PBS, and NaOH. Zheng's [17] approach to fabricate a microvessel scaffold will be adopted, in which two plexiglass pieces are sandwiched to form a functional scaffold [17]. The upper piece has rectangular microchannels inside the collagen matrix. This will be created by patterned PDMS stamp adopted from soft lithography methods, followed by a polymerization of the collagen gel on the patterned PDMS stamp inside the upper piece. The lower piece will contain a thin layer of collagen gel to enclose the microchannel in the upper piece. Dowel pins will be used to block the inlets and outlets from gel polymerization. Viscous finger patterning, used by Bischel et al, [18] is a method that will be used to ensure a more circular cross section of the lumen by exploiting the properties of two fluids with different viscosity [18]. After gel polymerization, HUVECs will be seeded into the microchannels at confluence to create an endothelium (see Figure 2). Appropriate pulsatile flow will be simulated by a perfusion system (see Figure 3). A peristaltic pump will be used to approximate shear stress by varying output pressure, calculated using computational models.

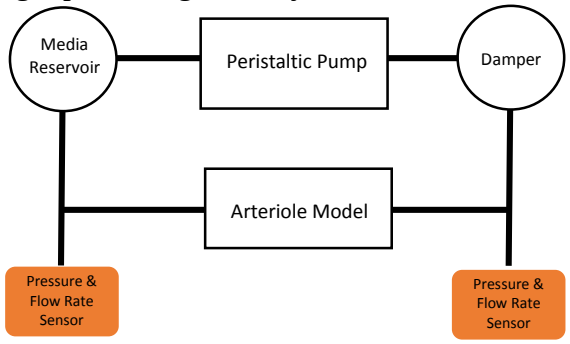

Figure 3 Schematic of perfusion system

System Validation: Three tests will validate the model's structure and properties. For histological testing, homogeneous tight junctions between CD31 stained ECs and α-SMA stained SMCs will be observed on 4μm tissue construct biopsies under an electron microscope. [15, 16] Selective permeabiltiy will be tested by pulsationally pumping fluorescent permeates along the vessel model under pressure that mimics in vivo blood flow. Flux of permeates across ECs into the SMC layer will determined. To test for biocompatibility, SMC with and without EC layers will be incubated in human platelet-rich plasma and observed for platelet adhesion on the EC absent control. [19]

Drug Testing: Three drugs will be administered to test cell signaling between EC and SMC layers in the model, measuring their effects by the changes in calcium ion concentration in SMCs. The

first two drugs will be relevant after structure and property validation and the third drug will act as a control until the model is integrated with the nervous and ocular systems. Nitroglycerin is a nitrodilator that binds non-specifically to ECs. It reacts with thiol to form an intermediate S-nitrosothiol and decomposes to liberate nitric oxide (NO). NO diffuses into adjacent SMCs and promotes formation of cyclic guanosine monophosphate (cGMP), which promotes muscle relaxation and an increase in blood calcium ion level. [20] Diazoxide is a potassium channel activator that binds specially to ECs. It inhibits membrane depolarization, switches off calcium ion channels and thus increase in blood calcium level. [21] The third drug is practolol, a selective β2 blocker with hypotensive effects. Practolol competes with catecholamines for binding at sympathetic receptor sites on ECs. It inhibits the release of neurotransmitters and contractile activities signaled by the sympathetic nervous system. [22] Practolol has proven safe for rats, monkeys, dogs, and pregnant rabbits, [23, 24, 25] but induces cardiac failure and ocular toxicity in 10% of cases in humans. [26] After integration with the ocular system, prolonged administration of practolol in the arteriole model should induce keratinization and squamous metaplasia of the conjunctival epithelium, creating a cloudy and slightly foaming appearance. [27] Drug test data obtained will be extrapolated using the microfluidics module of COMSOL software [28] to accommodate for effects on pharmacokinetics due to flow differences in microfluidics on chip, and in vivo arterioles. In vivo pharmacokinetics extrapolated from the model will be compared to clinical data: 5-20mcg nitroglycerin acts within 2-5 mins., lasting for about 30 mins. [29]; 300mg bolus of diazoxide administered within 10-30 sec. creates an antihypertensive effect, wearing off in about 5 mins. [21]; hypotensive effects of 100mg practolol are maximal in 1-3 hours [30, 31].

DISEASE MODEL – ATHEROSCLEROSIS ON A CHIP

Specific Aim: To emulate a 3D in vitro model of an atherosclerotic artery

Hypothesis: Hypertension and hyperlipidemia will initiate atherogenesis and result in gradual thinning of blood flow due to plaque build-up, simulating a standard in vivo case.

Rationale: More than 1 in 4 deaths in the US are due to cardiovascular or cerebrovascular diseases [32], both arising from atherosclerosis (AS). Current literature lacks a holistic 3D in vitro model of atherogenesis (AG). [33] Thus, a micro-fluidic arteriole model provides a modular diagnostic tool for further studies of AG, plaque rupture, thrombosis, and treatment options.

Background: The first step of AG is absorption and retention of low-density lipoproteins (LDL) into the vessel's subendothelium (SE). [34, 35] Hyperlipidemia and endothelial dysfunction (ED) provide entry points for LDL particles and are linked to an AS predisposition [36]. Upon absorption, LDL particles are oxidized (oxLDL) by inflammatory enzymes. [37, 38] OxLDL and LDL then trigger the release of proinflammatory lipids stimulating the chemokine release and expression of vascular cell adhesion molecules (VCAMs) on ECs. [34, 37], This recruits monocytes from blood to the ECs [37, 39], which infiltrate the intima, differentiate into macrophages, and engulf the toxic oxLDL, turning them into "foam cells." The accumulation of foam cells in a necrotic core induces formation of a fibrous collagen/SMC cap over atherosclerotic plaque, completing AG. [37]

Design: Applying hypertension to the vessel will initiate an increased response of vascular smooth muscle growth and a degradation of the endothelium, exposing the basal lamina and simulating ED. [40] Moreover, injecting oxLDL (LDL derived from healthy human volunteers, incubated with oxidizing agent) directly into the SE can accelerate foam cell formation. [41] Inducing LDL

weight dimension well above the accepted range will model hyperlipidemia. [42] CT-angiography examination will monitor the progression of plaque development.

Next Steps: Phase two will include a seeding of adventia to better simulate a medium/large artery which are more susceptible to atherosclerosis. Additionally, elastin-deficient areas of arteries are more prone to AG because elastin inhibits the penetration of LDL into the SE. Since the entire model lacks elastin, AG will not be as localized as in vivo cases. Once phase one proves to be a valid proof of concept, synthetic elastin will be added. Lastly, an array of VCAM-specific antibodies and immunofluorescence microscopy will monitor AG progression.

INTEGRATION OF SYSTEMS

The perfusion system in the arteriole and bone marrow model will be integrated with other systems by running the inlets and outlets of all systems in parallel. The HSCs in the bone marrow will differentiate into various WBCs and will be carried to the atherosclerosis disease model through shared perfusion medium. The WBCs may also be used within the tissue construct or the disease models of other systems (e.g using cadherins or cytokines). The SMC of arteriole model will respond to the aforementioned drug upon integration with the nervous system and side effects will be observed upon integration with an ocular system. Depending on the construct of other organ-on-a-chip systems, the arteriole and marrow offer dynamic physiology due to drugs or proteins released into the perfusion fluid shared between systems. A challenge is to ensure that the circulating medium is compatible with all integrated systems.

C – TECHNICAL RISKS

Immune System: Since the proposed approach is novel and untested, it is uncertain whether the HSCs will successfully proliferate and differentiate on chip as they would *in vivo*. Furthermore, the concentrations and frequency of growth factor perfusion during the 4 weeks will have to be calibrated. Proliferated blood cells pose challenge during integration as they may coagulate when flowing through microchannels into other systems, requiring further anticoagulation strategies.

Cardiovascular System: Implementing design changes to the arteriole may be difficult upon construction, as the outer structure of the design will be created via injection molding (changes require renewed molding). There is also an elaborate protocol to assemble the design together with the PDMS molds. A flexible plexiglass design from injection molding is a possible alternative. Additionally, hypertension may not be sufficient to initiate atherogenesis, in which oxLDL injection [41] or smoking-related toxin damage [43] to the endothelium will be required.

D – DELIVERABLES

Phase one deliverables will be implementing the two on-chip systems, with the CBC and blood differential tests done off-site. Phase two will involve integration of the CBC and blood differential tests on a chip using the proposed method, seeding of adventia to simulate a medium-sized artery, and the addition of synthetic elastin to the artery model. After the fabrication of proposed designs, a report of any updated changes will be provided. A data sheet of the properties (e.g. permeability of endothelium) for the proposed arteriole design will be delivered.

E – SCHEDULE

Time (mo.)	Immune System	Time (mo.)	Cardiovascular System
0-10	- Successful culture of MSC in peptide hydrogel 3D construct - Fabrication of PDMS chip	***0-6***	- Fabrication of microfluidic chip (e.g. fabrication of top and bottom plexiglass with patterned channels and polymerized collagen gel) - Monocultures of HUASMCs and HUVECs in vitro - Fabrication of top and bottom plexiglass without PDMS stamps to create channels and collagen gel.
10-18	- Successful co-culture of HSC into the 3D construct - Culture chamber integration into organ on-a-chip platform		
19-26	- Determination of perfusion growth factors		
26-40	- Successful mimicry of niche - System functionality validation (offline complete blood count)	***6-18***	- Successful co-culture of HUASMCs and HUVECs into the microfluidic chip (e.g. ensuring a confluent layer of ECs and proportional number of HUASMCs) - Fabrication of circular lumen via viscous finger patterning
		18-30	-Blood Compatibility tests, permeability of endothelium comparisons, and Cell histological tests
40-55	- Drug testing - Drug response analysis - Pharmacokinetics validation - Human physiology extrapolation	***30-42***	- Vasodilator and Vasoconstrictors testing
		42-55	- Disease model testing
55-60	Integration with other systems		

F – TEAM MEMBERS

The principle investigator for this program is Dr. Michael V. Sefton. The bone marrow model is designed Dr. Donald Ingber (*Afiq Asri*), leading scientist for Biomimetic Microsystems platform at the Wyss Institute at Harvard University. Cardiovascular system design is led by Dr. Ying Zheng (*Hei Yu Cheung*) whose research areas include microvascular remodeling, blood-endothelium interaction, and regenerative tissue engineering. Cardiovascular system validation is led by Prof. Hyung Joon Chun (*Candy Lam*), director of Yale's Pharmacy Building Fellow's Cardiovascular Clinic, whose research interest includes vascular pharmacology, GPCR signaling, and endothelial cell functions. Pharmacokinetic analysis will be executed by Dr. Michael Shuler (*Andrew Wong*) from Cornell University. He is an expert in pharmacokinetics-pharmacodynamics analysis on microfluidic chips. The disease model design is led by Dr. Subodh Verma (*Mohammad Saleh*), first and current Canada Research Chair in Atherosclerosis, whose research interests include inflammation, endothelial dysfunction, heart failure and atherosclerosis from benchtop to bedside. Next steps for immune system design validation is led by Dr. Yu-Chong Tai who is an expert in MEMS technology and integrated microelectronics, which will be essential in integrating CBC and blood differential counts into the system.

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