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Recreating complex vascularized tissue microenvironments in vitro

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The cost of bringing a new drug to market now exceeds $1 billion. The two most costly steps are pre-clinical testing of thousands of compounds to assess potential efficacy and side effects, and phase III clinical trials in humans. Only about 10% of drugs entering clinical trials make it to market, with 50% of drug candidates failing in expensive phase III trials due to lack of either safety or efficacy. We believe that reducing the cost and improving the success rate of drug discovery and validation will depend strongly on our ability to both rapidly and cost-effectively screen compounds, and to better predict success in large-scale human trials. Chemotherapeutics to treat cancer, which typically have a shorter time to market, are particularly prone to failing in phase III trials. Part of the problem is that drug screening platforms currently do not reflect the complex 3-dimensional nature of actual human organs.

To address this need we have created the 3D Vascularized Micro-Organ (VMO) platform. This consists of tissue-specific cells growing in a 3D matrix that receive nutrients and drugs through a network of living capillaries, just as they do in the body. In essence we have created “organs-on chips.” Up to 12 VMOs can be arrayed on a hand-sized plate and no pumps or specialized equipment are needed to support the device. This unique platform can be customized to include, for example, cardiomyocytes, pancreatic islets, neurons, or a variety of tumor cells (the Vascularized Micro-Tumor, or VMT, platform). The response of the vasculature itself to challenges can, of course, also be studied. The platform is transparent and so cell health and growth can be easily monitored over time.

The VMO and VMT platforms provide the most physiologic in vitro tissue systems currently available for drug evaluation.

Short Biography

Christopher Hughes

NAME: Hughes, Christopher Charles William
POSITION TITLE: Founder, 4Design Biosciences; Professor and Chair, UCI

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
University College, London	B.Sc. (Hon)	06/1981	Biochemistry
Institute of Psychiatry/KCH, University of London	Ph.D.	05/1988	Neuropathol./Vasc. Biol.
Brigham & Women's Hosp. Harvard Med. Sch.	Postdoctoral	07/1991	Vascular Biology

A. Personal Statement

I have extensive experience in the field of vascular biology and the process of angiogenesis. In particular, my lab has studied the role of notch signaling in vascular development and we were the first to demonstrate the role of notch in regulating tip cell function during angiogenic sprouting. We have recently published on the role of the transcription factor slug in angiogenesis, and are working on wnt regulation of vascular assembly. My lab developed the widely-used fibrin/bead assay of angiogenic sprouting. In collaboration with Drs. Steve George and Abe Lee we have also been focusing on novel drug-screening platforms, including "Vascularized Micro-Organ" microfluidic devices, and are co-founders of 4Design Biosciences, LLC. I have a joint appointment in Biomedical Engineering, and am Interim Director of the Edwards Lifesciences Center for Advanced Cardiovascular Technology. I am co-director of the OncoImaging and Biotechnology (OIB) Program – part of the NCI-designated Comprehensive Cancer Center at UCI – and Chair of the Department of Molecular Biology & Biochemistry. I was recently elected a Fellow of AAAS. I have mentored 15 Ph.D. students doing their thesis work in my lab, numerous rotation students, and several post-doctoral fellows. Several of these are now running their own research programs. Since founding 4Design Biosciences I have worked to develop relationships with industry and as a result we now have a contract with AstraZeneca to test new drugs. The background I have in vascular biology, bioengineering and entrepreneurship makes me ideally suited to contribute to this project.

B. Positions and Honors

Positions and Employment

1986-1987	Visiting Fellow, INSERM U26, Paris
1988-1990	Research Fellow, Dept. of Pathology, Brigham and Women's Hospital and Harvard Med. School
1990-1991	Instructor in Pathology, Brigham and Women's Hosp. and Harvard Medical School
1991-1995	Associate Research Scientist, Yale University School of Medicine

1996-2001	Assistant Professor, University of California, Irvine
2001-2006	Associate Professor (with tenure), University of California, Irvine
2003-2006	Associate Professor joint appointment in Biomedical Engineering
2006-	Professor, University of California, Irvine
2006-	Professor joint appointment in Biomedical Engineering
2009-	Chair, Department of Molecular Biology & Biochemistry, University of California, Irvine
2013-	Chair, HHT Foundation International Global Research and Medical Advisory Board
2014-	Interim Director, Edwards Lifesciences Center for Advanced Cardiovascular Technology
2014-	Co-Founder, 4Design Biosciences, LLC.

Other Experience and Professional Memberships

1994-	Member, North American Vascular Biology Organization
2006-2011	Co-Director Growth Factors and Signaling Program, Chao Family Comp. Cancer Center
2011-	Member, American Society for Investigative Pathology
2011-	Co-Director OncoImaging and Biotechnology Program, Chao Family Comp. Cancer Center
2012-	Member, Biomedical Engineering Society
2014-	Member AACR

Honors

1986	INSERM Visiting Fellowship, U26, Paris.
1988	American Heart Association Post-doctoral Fellowship
1993	Rapporteur: Inflammation in cardiovascular, lung, blood diseases. "Frontiers in Basic Sciences" National Heart, Lung, Blood Institute
1998	Young Investigator Award – Vascular Biology
2001	Pathology A study section, NIH, Ad Hoc
2001	NIH/NHLBI Special Emphasis Panel
2002-2006	American Heart Association, Western Affiliates, Study Section
2003	National Academies Keck Futures Initiatives Conference, Irvine CA
2005	EMBO Workshop on Notch signaling in development and cancer. Rome, Italy. Invited participant
2006	Cardiovascular Differentiation and Development (CDD) Study Section, NIH, Ad Hoc
2007-2011	CDD Study Section, NIH. Permanent Member
2010-2011	CDD Study Section, NIH. Chair
2008-2010	NIH Special Emphasis Panel
2010	Hereditary Hemorrhagic Telangiectasia (HHT) Foundation International – Chair, Committee on Future Research Directions
2011	NIH Special Emphasis Panel, Co-Chair
2011-	HHT Foundation International – Global Research and Medical Advisory Board member
2011-	Editorial Board – Angiogenesis
2011	UCSF – 3rd Annual Allison Raaen Lectureship
2013	CDDT Study section. NIH. Ad Hoc
2014	Co-organizer, HHT session Chair, NAVBO meeting
2015	Fellow, AAAS – American Association for the Advancement of Science
2015	Co-Organizer, HHT International Meeting, Florida.

2015-	Councilor – North American Vascular Biology Association
2015-2016	Program Committee for the International Vascular Biology Meeting (IVBM)
2016-	Co-Organizer, HHT2017 International Meeting

C. Contribution to Science

(in rough chronological order of when work started)

Over 80 peer-reviewed research publications, 9 reviews and 7 book chapters. H-index = 47

1. In vitro models of the vasculature.

I and my lab have developed several models that are now in widespread use. My early work focused on developing a blood-brain barrier model, and we used this to study the immunological properties of brain endothelium. More recently we established the fibrin/bead assay, which allows for the development of fully lumenized angiogenic sprouts in culture. To date, all of the key observations we and others have made with this system have subsequently been validated by us and others *in vivo*, mostly in mice.

a. Male DK, Pryce G, Hughes CCW. 1987. Antigen presentation in brain: MHC induction on brain endothelium and astrocytes compared. Immunology 60:453-459 [PMID: 3106198]

b. Hughes CCW, Male DK, Lantos PL. 1988. Adhesion of lymphocytes to cerebral microvascular cells: Effects of interferon-□, tumour necrosis and interleukin-1. Immunology 64:677-681 [PMID: 3139550]

c. Nakatsu MN, Sainson RCA, Aoto JN, Taylor KL, Aitkenhead M, Pérez del Pulgar S, Carpenter PM, and Hughes CCW. 2003 Angiogenic sprouting and capillary lumen formation modeled by human umbilical vein endothelial cells (HUVEC) in fibrin gels: the role of fibroblasts and Angiopoietin-1. Microvascular Research 66: 102-112 [MVR most downloaded article for 2003] [PMID: 12935768]

d. Nakatsu MN and Hughes CCW. 2008. An optimized three-dimensional *in vitro* model for the analysis of angiogenesis. Methods in Enzymology 443: 65-82 [PMID: 18772011]

2. Endothelial-T cell interactions.

My work as a post-doc and early stage investigator at UC Irvine focused on the role of endothelial cells (EC) in presenting antigen to memory T cells. We defined key costimulatory molecules expressed by EC, and confirmed that EC could activate T cells without the assistance of other immune cell types, using single cell analysis of cytokine expression. We also identified a CsA-resistant pathway of T cell activation by EC, and showed that trans-EC migration was wnt-dependent. In 2004 we published an influential review on differences between mouse and human immunology that still garners over 75 citations a year.

a. Hughes CCW, Savage COS, Pober JS. 1990. Endothelial cells augment T cell IL-2 production by a contact-dependent mechanism involving CD2:LFA-3 interaction. J. Exp. Med. 171:1453-1467 [PMID: 1692079]

b. Karmann K, Pober JS and Hughes CCW. 1994. Endothelial cell-induced resistance to cyclosporin A in human peripheral blood T cells requires contact-dependent interactions involving CD2 but not CD28. J. Immunol. 153: 3929-3937 [PMID: 7523510]

c. Wu B, Crampton SP, and Hughes CCW. 2007. Wnt signaling induces MMP expression and regulates T cell transmigration. Immunity 26: 227-239. [PMID: 17306568]

d. Mestas J, and Hughes CCW. 2004. Of mice and not men: differences between mouse and human immunology. J. Immunol. 172: 2731-2738 [PMID: 14978070] (cited over 1300 times)

3. Notch regulation of angiogenesis.

In 1996 my new lab began working on gene regulation during angiogenesis. We identified a transcription factor, HESR1 (HEY1), that operates downstream of Notch, and were the first to show that it regulates angiogenesis. We were also the first to show that HESR1 regulates VEGFR2 expression, and that Notch is a regulator of angiogenic tip cell function. A ligand for Notch, Jagged-1 is induced by TNF in EC, and we showed that this regulates inflammatory angiogenesis.

a. Henderson AM, Wang S-J, Taylor AC, Aitkenhead M and Hughes CCW 2001 The bHLH transcription factor HESR1 regulates endothelial cell tube formation. J. Biol. Chem. 276: 6169-6176 [PMID: 11069914]

b. Taylor KL and Hughes CCW. 2002. Notch activation during endothelial cell network formation in vitro targets the basic HLH transcription factor HESR1 and downregulates VEGFR-2/KDR expression. Microvascular Research. 64: 372-383 [PMID: 12453432]

c. Sainson RCA, Aoto J, Nakatsu MN, Holderfield M, Conn E, Koller E, and Hughes CCW. 2005 Cell autonomous Notch signaling regulates endothelial cell branching and proliferation during vascular tubulogenesis. FASEB J 19: 1027-9 [PMID: 15774577]

d. Sainson RCA, Johnston DA, Chu HC, Holderfield MT, Nakatsu MN, Crampton SP, Davis J, Conn E, and Hughes CCW. 2008 TNF Primes Endothelial Cells for Angiogenic Sprouting by Inducing a Tip Cell Phenotype. Blood 111: 4997-5007. [PMID: 18337563]

4. Molecular regulation of angiogenesis and HHT.

We have performed several screens for angiogenesis-regulated genes and identified numerous key molecules, including transcription factors, cell-surface receptors and extracellular matrix proteins. We have defined a critical role for matricellular protein expression by fibroblasts in angiogenesis and have uncovered a role for IFITM1 in regulating lumen formation. Most recently we have defined a role for slug and snail in controlling the EMT-like process of angiogenic sprouting. We have studied the role of the ALk1 ligand BMP9 in regulating angiogenesis and collaborated on the identification of an HHT modifier gene.

a. Kim J-H, George SC, and Hughes CCW. 2012. BMP9 induces EphrinB2 expression in endothelial cells through an Alk1-BMPRII/ActRII-ID1/ID3-dependent pathway: implications for hereditary hemorrhagic telangiectasia type II. Angiogenesis 15: 497-509 [PMID: 22622516]

b. Benzinou M, Clermont FF, Letteboer TGW, Kim J-H, Luu MT, Harradine KA, Roy R, Espejel S, Quigley D, Zaid M, Aouizerat BE, Ploos van Amstel JK, Giraud S, Dupuis-Girod S, Lesca G, Plauchu H, Hughes CCW, Westermann CJJ, and Akhurst RJ. 2012. A novel mouse to human genetics strategy identifies *PTPN14* as a genetic modifier of arterio-venous malformation in HHT. Nature Communications 3: 616 doi: 10.1038/ncomms1633 [PMID: 22233626]

c. Popson SA, Ziegler ME, Chen X, Holderfield MT, Shaaban CI, Fong AH, Welch-Reardon KM, Papkoff J, and Hughes CCW. 2014. Interferon-induced transmembrane protein 1 regulates endothelial lumen formation during angiogenesis. Arterio. Thromb. Vasc. Biol. 34: 1011-19 [PMID: 24603679]

d. Welch-Reardon KM, Ehsan SM, Wang K, Newman AC, Romero-Lopez M, Fong AH, George SC, Edwards RA, Hughes CCW. 2014 Angiogenic sprouting is regulated by endothelial cell expression of Slug (Snai2). J. Cell. Sci 127: 2017-2028 [PMID: 24554431]

5. Microphysiological Systems.

In a highly successful collaborative effort with Dr. Steve George and Dr. Abe Lee we have created a unique platform that incorporates perfused human vasculature in culture. The vessels carry tissue culture medium and can support the growth of various tissues in 3D including cardiomyocytes, bone-marrow-derived cells, and tumor cells. Current work is developing this into a high-throughput screening platform for anti-cancer drug discovery, and we are in the final stages of preparing a manuscript validating the platform for this purpose. We have also worked with Dr. George to create a model of tumor cell intravasation.

a. Moya ML, Hsu, YH, Lee AP, Hughes CCW, and George SC. 2013. In vitro perfused human capillaries. Tissue Engineering 19: 730-737 [PMID: 23320912]

b. Hsu YH, Moya ML, Hughes CCW, George SC and Lee AP. 2013 A microfluidic platform for generating large-scale nearly identical human microphysiological system arrays. Lab on a Chip 13: 2990-2998 [PMID: 23723013]

c. Wang X, Phan DT, Sobrino A, George SC, Hughes CCW, Lee AP. 2016. Engineering anastomosis between living capillary networks and endothelial cell-lined microfluidic channels. Lab on a Chip 16 (2): 282-290 [PMID: 26616908]

d. Sobrino A, Phan DT, Datta, R, Wang X, Hachey SJ, Romero-Lopez M, Gratton E, Lee AP, George SC, and Hughes CCW. 2016 3D microtumors *in vitro* supported by perfused vascular networks. Nature Scientific Reports 6: 31589 [PMID: 27549930]

URL for published work:
https://scholar.google.com/citations?user=C2daMJ4AAAAJ&hl=en

D. Research Support (Academic)

Ongoing Research Support

R01 CA180122-01 (Hughes, PI) 08/01/13-07/30/17
PQD5 Development of in Vitro Vascularized Microtumors for Drug Screening
The goal of this project is to develop an *in vitro* platform for drug screening that incorporates a microtumor supported by perfused human vasculature.
Role: PI

R01 CA170879-01 (George) 06/01/12-05/31/16 (NCE)
PQ24 A 3-D in vitro platform of tumor metastasis
The goal of this proposal is to create a microfluidic platform suitable for studies of metastasis. This is not a drug-screening platform, but rather, is designed to capture tumor cells as they metastasize *in vitro*.
Role: Co-PI

UC4 DK104202-01 (Sander) 08/01/14-07/31/19
A 3D biomimetic human islet to model beta cell function in health and disease
The goal of this proposal is to create an *in vitro* biomimetic of the human pancreases using microfluidic and 3D culture technology.
Role: Co-PI

R01 HL122869-01 (Akhurst) 09/01/15-05/31/19
Circulating cells as tools to study vascular pathobiology of HHT
The goal of this proposal is to investigate the role of circulating endothelial precursor cells in the etiology of HHT
Role: Co-PI

UH3 TR-000481 (George) 08/01/14-07/30/17
An *in vitro* model of perfused tumor and cardiac tissue
The central objective is to use microfluidics to create an *in vitro* 3D system of perfused human cardiac tissue in line with a human tumor so as to provide a platform for investigating potentially cardiotoxic anti-neoplastic drugs.
Role: Co-PI

R21 EB022704 (Hughes, PI) 07/01/16-06/30/18
Real-time bioluminescent detection of circulating biomarkers
The goal of this project is to generate genetically-modified cells that can respond to changes in insulin concentration by emitting light. These will be incorporated into microfluidic devices to monitor pancreatic beta cell health.
Role: PI

T32 HL116270 (Hughes, PI) 06/01/13-05/31/18
Translational cardiovascular technology training program
The goal of this T32 is to train students in cardiovascular biology, related bioengineering principles, entrepreneurship to encourage entry into both academic and industry/start-up arenas.
Role: PI

P30 CA62203-18 (Van Etten) 07/01/15-06/30/20
Cancer Center Support Grant
This grant supports the NCI-designated Chao Family Comprehensive Cancer Center at the University of California, Irvine.
Role: Co-Investigator