ENDOTHELIAL PROGENITOR CELLS, URAEMIC TOXINS, & THE DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION IN CHRONIC KIDNEY DISEASE

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Submitted in fulfillment of the

Degree of Doctor of Philosophy,
University of Adelaide,
June 2012

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Thesis Abstract

Morbidity and mortality rates for cardiovascular disease (CVD) are increased among end stage kidney disease (ESKD) patients receiving dialysis treatment, and not corrected with kidney transplantation (KTx). Classic CVD risk factors do not fully predict the increased risk, with novel factors causing endothelial dysfunction (ED), leading to arteriosclerosis, congestive heart failure (CHF) and sudden death, key to disease pathogenesis.

These novel factors include bone marrow (BM) derived endothelial progenitor cells (EPCs), which have key roles in maintenance, repair and growth of the endothelium. There is limited data about the role of EPCs and CVD in the ESKD population. This uraemic milieu includes p-cresol (sulfate, PC/S) and indoxyl sulfate (IS), toxins associated with CVD in ESKD.

In this thesis, the relationship between CVD and ESKD, and the potential role of EPCs and uraemic toxins was examined from epidemiological, clinical and laboratory perspectives.

Data was obtained for the period between 2002-2007 for all hospital separations in Australia. Analysis was performed based on ICD-9/10 coding. This showed (for the first time in an Australian population): (i) an increase in risk for CVD hospital separations among dialysis and KTx, with higher rates for CHF than acute cardiac events (ACE); (ii) an advantage for KTx recipients in regards to ACE, but not CHF hospital separations, over dialysis recipients, and (iii) for CHF, no increase in in-hospital mortality, or length of stay per separation for any ESKD group compared to controls.

At a clinical level, in groups of haemodialysis (HDx), KTx patients and controls, low peripheral blood (PB) EPC numbers were correlated with surrogate markers of CVD

and ED. No clear relationship of IS and PC/S with ED was seen (although study power was limited).

For *in vitro* studies, techniques were developed for isolation (Flow sort and AutoMACS), enumeration (FACS) and culture expansion of EPCs from BM and umbilical cord blood samples.

The effects of uraemic serum and toxins PC and IS on cultured endothelial cells (ECs) and EPCs *in vitro* was examined, as a model of vascular pathology in ESKD.

Greater HUVEC VCAM-1 expression and reduced tube formation in Matrigel were observed in response to increasing PC concentration than IS. The effect of IS (but not PC) at higher concentration in Matrigel was reduced by the addition of EPCs. Akt/ERK expression by western blot, cell migration to VEGF, and supernatant investigation by FlowCytoMix for soluble cell surface markers, were also performed. Testing of HUVEC function post-exposure to sera from control, transplant and HDx recipients did not replicate the above results on the basis of sera PC and IS levels.

In summary, this thesis has explored the increased burden of CVD in ESKD patients in Australia, the relationship of EPCs, both *in vivo* and *in vitro,* to vascular disease in this setting, and the role of uraemic toxins as agents for CVD. These results underline why certain therapies may not be effective in the ESKD population for CVD prevention, and suggest novel approaches are needed.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Dr Shaundeep Sen and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Acknowledgements

This thesis began as a very loose collection of ideas, which over time, developed into more solid plans. The initial, more grandiose, thoughts were tempered, whilst blind optimism was quickly replaced by pessimistic reality, and then later guarded hope.

I was neither aware nor prepared for what a PhD really required of it's aspirant, until well into my candidature – for a physician, clinical work can be heavy, but usually provides you with regular positive feedback. Basic and clinical research do not provide the same consistent gratification, despite greater effort.

As my principal supervisor, Stephen McDonald has been a mentor, motivator, teacher and friend. He has forged a career that inspires me to do more with my own. I cannot thank him enough, especially for his gentle, and at other times more vigorous, encouragement, as well as his consistent broad vision for the thesis.

Toby Coates I am also privileged to consider a friend, as well as mentor. His balance of a heavy clinical load, whilst directing a large active laboratory is testament to his unlimited energy, whilst his personal generosity is renowned. I thank him in particular for his ongoing enthusiasm for my work, and unwavering optimism.

Claudine Bonder became key to my completion of this PhD. As a scientist, she has had to put up with my non-existent laboratory skills and irregular work hours, without once making me feel out of place in the lab. As a manager, Claudine shows how a very successful research program can be developed in the midst of multiple competing factors for your time. For her friendship, I will be always grateful.

Beyond my supervisors, I received significant support from several different groups of individuals. I wish to acknowledge from the cardiology department at the QEH Dr Alicia Chan for the initial EPC isolation, and Prof John Beltrame and Dr Christine Bell. From Toby's lab Emma Leedham, Clyde Milner, Chris Drogemuller, Claire Jessup, Matthew Stephenson and Svjetlana Kireta, and all the PhD students and staff, who made my time there such a joy. From Claudine's lab, Kiwi Sun (who constantly inspires me with her work ethic), Samantha Escarbe, Sarah Brice, Michaelia Cockshell, Katie Tooley, Kate Parham, Jyotsna Pippal and Jeff Barrett. From the Detmold Imaging Suite, Katherine Pilkington and Ghafar Sarvestani, Bin Tong from the AIHW, and Dr Joachim Jankowski from the Charite.

I would also like to thank all the physicians, nurses, administrators, patients and subjects involved in the clinical study, in particular Inspector Philip Newitt for his recruitment drive, and the staff of the renal units at the QEH, RAH and FMC.

My family has continued to support, encourage and inspire me throughout my life and my career, without question. I gained daily inspiration from the friendship and love given to me by my parents Pradeep and Surekha, sister Rukmi, parents-in-law John and Carole, brother-in-law Andrew, and sisters-in-law Catherine and Benita, who have all individually achieved so much.

Finally, to my wonderful wife Sarah and our gorgeous children Abbie and Aidan, I dedicate this work. They have dealt with my low times, infuriating procrastination, long hours in the lab, and absences from home. They have always supported, encouraged and loved me, and have been a constant reminder of what is valuable and important in life. Without Sarah, and the sacrifices she in particular has made, none of this would have been possible.

Honours and Awards

2010 Queen Elizabeth Hospital

- Departmental Postgraduate Research Scholarship

2009 Transplantation Society of Australia and New Zealand Annual Scientific Meeting

- Kidney Health Australia Prize for the Best Presentation in the Field of Clinical Research
- Young Investigator Award

2009 Queen Elizabeth Hospital Research Day

- Best Poster Prize

2007 Kidney Health Australia

- PhD Biomedical Scholarship

Publications

Penko D, Mohanasundaram D, Sen S, Drogemuller C, Mee C, Bonder CS, Coates PTH, and Jessup CF. Incorporation of endothelial progenitor cells into mosaic pseudoislets. Islets. 3(3): 73-79. May/June 2011

Sen S, McDonald SP, Coates PTH and Bonder CS. Endothelial Progenitor Cells: novel biomarker and promising cell therapy for cardiovascular disease. Clinical Science. 120(7): 263-83, 2011 April

Conference Presentations

2011 – American Society of Nephrology: Renal Week/ASM

- S Sen, PTH Coates, CS Bonder, SP McDonald. "The interaction of uraemic toxins and endothelial progenitor cells in the progression of cardiovascular disease" – Poster

2011 - Australia and New Zealand Society of Nephrology: ASM

- S Sen, PTH Coates, CS Bonder, SP McDonald. "The interaction of uraemic toxins and endothelial progenitor cells in the progression of cardiovascular disease" – Poster

2010 – The Transplantation Society: World Congress

- S Sen, SP McDonald. "Kidney Replacement Therapies are Associated with Increased Rates of Hospital Separation and Mortality for Acute Cardiac Events" – Mini-Oral

2009 – Queen Elizabeth Hospital Research Day

- S Sen, B Tong, SP McDonald. "Kidney Replacement Therapies are Associated with Increased Rates of Hospital Separation and Mortality for Acute Cardiac Events" – Poster

2009 – Australia and New Zealand Society of Nephrology

- S Sen, B Tong, SP McDonald. "Kidney Replacement Therapies are Associated with Increased Rates of Hospital Separation and Mortality for Acute Cardiac Events" – Poster
- S Sen, SP McDonald, PTH Coates, CS Bonder. "In vitro Endothelial Cell Tube-Forming Capacity Reduced By Transplant Sera, But Migration Increased by Haemodialysis" – Poster

2009 – Transplant Society of Australia and New Zealand

- S Sen, SP McDonald, PTH Coates, CS Bonder. "A Novel Protocol for the Expansion of CD133+ Human Endothelial Progenitor Cells in EGM-2 Plus Cytokines" – Oral Presentation
- S Sen, B Tong, SP McDonald. "Renal Transplantation Decreases Rates of Hospital Separation for Both Acute Cardiac Events and Cardiac Failure Compared to Dialysis" – President's Prize Session Oral Presentation

Manufacturers

- Abbott Ireland, Longford, Co. Longford, Ireland
- AtCor Medical Pty Ltd, West Ryde, NSW, Australia
- Baxter Healthcare, Old Toongabbie, NSW, Australia
- Dr B. P. Bailey, Lane Cove, NSW, Australia
- BD, Franklin Lakes, NJ, USA
- BD Biosciences,
    - San Diego & San Jose, CA, USA
    - North Ryde, NSW, Australia
- Beckman Coulter, Miami, FL, USA
- Bender MedSystems,
    - Burlington, CA, USA
    - Vienna, Austria
- Biomedical Technologies, Stoughton, MA, USA
- Bio-Rad Laboratories, Hercules, CA, USA)
- Carl Zeiss, Gottingen, Germany
- Cell Signalling, Danvers, MA, USA
- Corning Life Sciences
    - Acton, MA, USA
    - Corning, NY, USA
- Detmold Family Trust Cell Imaging Facility, Hanson Institute, SA Pathology
- Diagnostica Stago Inc., Parsippany, NJ, USA
- Fresnius, Kabi, Norge

- FujiFilm Life Sciences, Stamford, CT, USA
- GE Healthcare, Buckinghamshire, UK
- Gibco Invitrogen, Gaithersburg, MD, USA
- GlaxoSmithKline, Boronia, Vic, Australia
- GraphPad Software Inc, California USA
- Greiner Bio-One, Kremsmunster, Austria
- Hyclone, Utah, USA
- Institute for Medical and Veterinary Science (IMVS) / SA Pathology, Adelaide, SA, Australia
- Invitrogen / Molecular Probes, Eugene, OR, USA
- Lonza, Walkersville, MD, USA
- MacoPharma, Mouvaux, France
- Microsoft Corporation, Redmond, WA, USA
- Miltenyi Biotech, Bergisch Gladbach, Germany
- Olympus, Mount Waverly, Vic, Australia
- Olympus UK Ltd, Southend-On-Sea, Essex, UK
- R&D Systems, Minneapolis, MN, USA
- Roche Diagnostics GmbH, Mannheim, Germany
- Santa Cruz, Santa Cruz, CA, USA
- Sarstedt, Mawson Lakes, SA, Australia
- Siemens Healthcare Diagnostics, Deerfield, IL, USA
- Sigma Aldrich, St Louis, MO, USA
- Sigma Pharmaceuticals, Croydon, Vic, Australia
- SonoSite Inc, Bothwell, WA, USA

- Southern Biotech, Birmingham, AL, USA
- StataCorp LP, Texas USA
- SupelCo, Bellafonte, PA, USA
- Sysmex America Inc., Mundelein, IL, USA
- Thermo Scientific, Waltham, MA, USA
- Vital Diagnostics, Bella Vista, NSW, Australia
- Wayne Rasbands, National Institutes of Health, USA
- Worthington Biomedical Corporation, Lakewood, NJ, USA

Abbreviations

AA	ascorbic acid
ABS	Australian Bureau of Statistics
ACE	acute cardiac events
Ac-LDL	acetylated low density lipoprotein
ACEi	angiotensin converting enzyme inhibitor
ADMA	asymmetric dimethylarginine
AI	augmentation index
AIHW	Australian Institute of Health and Welfare
AMI	acute myocardial infarction
Ang-1	angiopoietin I
ANZDATA	Australia and New Zealand Dialysis and Transplant Regsitry
AT I	angiotensin I
ATRA	angiotensin II receptor antagonist
bFGF	basic fibroblast growth factor
BNP	brain natriuretic peptide
BP	blood pressure
^{o}C	degrees Celsius
CAC	coronary artery calcification
CCA	common carotid artery
CCL	CC chemokine ligand
CFDA	carboxy-fluorescein diactetate
CHF	congestive heart failure
cIMT	carotid artery intima-medial thickness

CKD	chronic kidney disease
CK-MB	myocardial creatine kinase
CMV	cytomegalovirus
CPC	circulating progenitor cell
CRP	C-reactive protein
CT	computerised tomography
cTnT	cardiac troponin T
CVD	cardiovascular disease
CXCL	CXC chemokine ligand
CXCR	CXC receptor
Cy5/7	cyanine 5/7
DAPI	4’,6-diamidino-2-phenylindole
DBP	diastolic blood pressure
Dil Ac-LDL	1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-acetylated-low density lipoprotein
DMSO	dimethylsulfoxide
DPP IV	dipeptidylpeptidase IV
EBCT	electron beam computerised tomography
EC	endothelial cell
ECG	electrocardiograph
ECGF	endothelial cell growth factor
ED	endothelial dysfunction
EDTA	ethylenediaminetetraacetic acid
eNOS	endogenous nitric oxide synthetase
EPC	endothelial progenitor cell

EPO	erythropoietin
ESKD	end-stage kidney disease
FACS	fluorescence activated cell sorter
FCS	fetal calf serum
FDR	flow debt repayment
FITC	fluorescein isothiocianate
FLK	Fms-like tyrosine kinase
G-CSF	granulocyte colony stimulating factor
GFR	glomerular filtration rate
GM-CSF	granulocyte macrophage colony stimulating factor
GTN	glycerol trinitrate
HbA1C	haemoglobin A1C
HDL	high density lipoprotein
HDx	haemodialysis
hEGF	human endothelial growth factor
HIF-1α	hypoxia inducible factor-1alpha
HMGB1	high-mobility box group 1
HPLC	high performance liquid chromatography
HT	hypertension
HUVEC	human umbilical vein endothelial cell
ICA	internal carotid artery
ICAM-1	intercellular adhesion molecule-1
ICD-10	International Classification of Disease, Version 10
Ig	immunoglobulin

IGF-1	insulin-like growth factor-1
IL	interleukin
IM	intramuscular
IS	indoxyl sulfate
KDR	kinase insert domain receptor
KRT	kidney replacement therapy
KTx	kidney transplant
LDL	low density lipoprotein
LV	Left ventricle
LVEF	left ventricular ejection fraction
LVH	left ventricular hypertrophy
MACS	magnetic activated cell separation
MAPC	multipotent adult progenitor cell
MCP-1	monocyte chemotactic protein-1
MMP	matrix metalloproteinase
MNC	mononuclear cell
MRA	magnetic resonance angiography
MRI	magnetic resonance imaging
m/s	metres per second
NO	nitric oxide
NFκB	nuclear factor kappaB
NIH	National Institutes of Health
NT-proBNP	N-terminal pro-brain natriuretic peptide
oxLDL	oxidised low density lipoprotein

PB	peripheral blood
PC	para-cresol, p-cresol
PCS	para-cresol sulfate, p-cresol sulfate
PDx	peritoneal dialysis
PE	phycoerythrin
PECAM-1	platelet endothelial cell adhesion molecule-1
PET	positron emission tomography
PMT	photomultiplier tubes
PPARγ	peroxisome proliferator activated receptor gamma
PTH	parathyroid hormone
PVD	peripheral vascular disease
PWA	pulse wave analysis
PWV	pulse wave velocity
RAGE	receptor for advanced glycation end products
RNA	ribonucleic acid
SBP	systolic blood pressure
SCF	stem cell derived factor
SDF-1	stromal cell derived factor-1
sICAM	soluble intercellular adhesion molecule
SMC	smooth muscle cell
SPECT	single photon emission computerised tomography
TGFβ-R1 inhIII	tissue growth factor beta R1 inhibitor III
TNFα	tumour necrosis factor alpha
UCB	Umbilical cord blood

US	ultrasound
USRDS	United States Renal Data Systems
VCAM-1	vascular cell adhesion molecule - 1
VE-cadherin	vascular endothelial cadherin
VEGF	vascular endothelial growth factor
VEGF-R2	vascular endothelial growth factor receptor 2
vWF	Von Willebrand factor