



**White Rose HIP  
Health Technology  
Bulletins**

The White Rose Health Innovation Partnership (WRHIP) aims to accelerate new health-related technologies by facilitating interactions between academia, industry and the NHS using an *open innovation* approach.

The new projects funded as part of this initiative are built upon a foundation of excellence in health innovation by the Partnership's members. This series of Health Technology Bulletins offer an introduction to this research excellence and cover a broad range of clinical and technology areas.

Each bulletin is written to give a general introduction to the topic area along with short case studies of clinical applications of new knowledge. Information is also presented on where to learn more about these new technologies and health challenges, and how to access the network of health innovation professionals established by the Partnership.

**Cardiovascular Translational Research:  
Challenges & Opportunities**

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Annually 50% of people in the Western world die from cardiovascular diseases (CVD). The European Society of Cardiology and the European Heart Network estimate the healthcare and other related costs of CVD to exceed €192 billion a year. Identifying areas of translational research that improve survival

and rehabilitation by early detection, improving patient stratification and monitoring treatment responses could ameliorate such human and financial burdens. Here we consider areas of clinical need and the challenges and opportunities that exist for cardiovascular research.

**Assessing the risk of atherosclerotic  
plaque rupture**

This is the 'million dollar' question. Patients normally present with signs and symptoms suggestive of an atherosclerotic plaque affecting the coronary, cerebral or peripheral circulation. Accurate prediction of the progression of such atherosclerotic lesions would allow clinicians to monitor and aggressively treat high-risk patients, to prevent clinical sequelae and improve prognosis. Although 'vulnerable' lesions display certain histological characteristics, identifying these

characteristics in a patient poses multiple technical hurdles. An atherosclerotic plaque's susceptibility to rupture is determined by the integrity of the fibrous cap and the balance of pro- and anti-inflammatory cytokines and growth factors (Table 1). The inflammatory cellular constituents of a plaque produce pro-inflammatory molecules, which can degrade the collagenous extracellular matrix and stimulate cellular apoptosis, destabilizing the atherosclerotic lesion.

**Table 1. The main mechanisms and factors associated with atherosclerotic plaque rupture.**

<b>Endothelial desquamation</b>	<b>Angiogenesis</b>	<b>Fibrous cap fracture</b>
<i>Endothelial cell death</i>	<i>Microvessel formation</i>	<i>Inhibition of collagen production</i>
<ul style="list-style-type: none"> <li>• Inflammatory mediators</li> <li>• Killer T-cells</li> </ul>	<ul style="list-style-type: none"> <li>• Basic fibroblast growth factor</li> <li>• Vascular endothelial growth factor</li> </ul>	<ul style="list-style-type: none"> <li>• Cytokines (interferon-<math>\gamma</math>)</li> </ul>
<i>Sub-endothelial basement membrane degradation</i>		<i>Collagen catabolism</i>
<ul style="list-style-type: none"> <li>• Inflammatory mediators</li> <li>• Oxidised lipoproteins</li> <li>• Matrix metalloproteinases (MMPs)</li> </ul>		<ul style="list-style-type: none"> <li>• Collagenases</li> <li>• Gelatinases (eg MMP-2 and MMP-9)</li> <li>• Decreased tissue inhibitors of MMPs (TIMPs)</li> </ul>



## Molecular imaging

Our increased understanding of specific cellular markers has provided novel targets for imaging modalities (magnetic resonance imaging [MRI], nuclear-computed tomography imaging, optical and ultrasound imaging). For example, recent studies have investigated direct imaging of macrophages, a key inflammatory mediator. *In vivo*, within 24-48 hours following administration, magnetic iron oxide nanoparticles accumulate in macrophages within inflammatory regions of an atherosclerotic plaque. This may provide a method to analyse inflammatory changes associated with vulnerable atherosclerotic lesions and hence predict 'at-risk' patients.

The number of apoptotic cells within an atherosclerotic lesion correlates with plaque instability. During apoptosis, cells express multiple cell-surface molecules. Using radio-labelled annexin A5, a high-affinity ligand for phosphatidylserine, investigators have successfully imaged cellular apoptosis in patients with acute myocardial infarction. Recently, in a small group of patients, an elevated radio-labelled annexin A5 nuclear signal was found in

the ipsilateral carotid lesion following a transient ischaemic attack ('mini-stroke'). Whilst such findings need to be validated in larger cohorts, they may offer a reliable method to risk-stratify patients.

Other potential targets include oxidized low-density lipoprotein, matrix metalloproteinases and endothelial cell markers such as vascular cell adhesion molecule-1, all of which are known to be abundantly present in vulnerable atherosclerotic plaques. Current techniques for imaging high-risk plaques are invasive and the limited targets lack the sensitivity and specificity to allow widespread and routine application. A strategy to improve detection of plaque vulnerability may be to target multiple markers rather than a single one, with the proposed imaging modalities. For example, supramagnetic iron oxide particles could be labelled with 2 or 3 different targeting molecules. The molecules and their relative proportions would require extensive study to maximize targeting sensitivity and specificity. The future of such technology could conceivably be a 'silver-bullet' approach: a single agent or particle that targets, images and acts as a drug-delivery vehicle for vulnerable atherosclerotic plaques (so-called 'multifunctional particles').

## Biomarkers

Significant advances in agent chemistry and imaging platforms have provided real progress in translational bench-to bedside studies. A notable limitation is the lack of 'point-of-care' applications of these imaging modalities. Most of the proposed applications require a time-lag of at least 24 hours for optimal visualization of high-risk atherosclerotic lesions. Multiple serum or plasma biomarkers have been identified and proposed as potential diagnostic adjuncts (Table 2); some may also have a role in monitoring prognosis and response to treatment. This is increasingly important in a healthcare environment where new and expensive treatments, such as the new anti-cancer agents, are being either rationed or denied to patients. With the growing trend of using proteomic assays such as mass spectrometry and proton-nuclear magnetic resonance spectroscopy (H-NMR) to assess biological samples, and a renewed interest in CVD, it is likely that new biomarkers will continue to be discovered. However, the majority of current biomarkers have not been integrated into routine clinical practice. Reasons for this include:

a lack of standardized, reproducible and reliable data; unvalidated reference ranges; assays have been deemed not cost-effective; limited sensitivity and specificity; inability to predict pathological changes and severity of the disease.

There are a number of approaches to address these important issues. Similar to the strategy outlined for imaging modalities, panels of biomarkers may be used to improve their efficacy and utility. A separate but controversial method would be to adopt a standardized approach for data collection and processing of samples for future prospective cohort studies. Such resources, with extensive reporting at enrolment and complete follow-up and survival data, would allow efficient screening of multiple factors. These studies would require the use of 'banked' samples, which poses many ethical problems in addition to the logistical issues of maintaining such a database. However, lessons should be taken from the Reduction of Atherothrombosis for Continued Health (REACH) registry, which successfully enrolled and followed-up 95% of over 68,000 patients worldwide.

**Table 2. Potential biomarkers for use in cardiovascular disease**

Inflammatory markers	→	C-reactive protein (CRP); serum amyloid A (SAA); interleukin 6 (IL-6); interleukin 1-β (IL-β); intracellular and vascular adhesion molecules (ICAM and VCAM); leukocyte count; cell surface markers (CD40L); additional cytokines and prostaglandins
Haemostatic and rheological markers	→	Fibrinogen; fibrin; D-dimer; antithrombin III; fibrinopeptide A; von Willibrand factor; tissue plasminogen activator (t-PA) antigen; factor VII; prothrombin time; prothrombin fragment 1 + 2; whole blood viscosity; plasma viscosity; haematocrit
Liquid associated markers	→	Lipoprotein(a); myeloperoxidase; low phospholipid transfer protein (PLTP); lectin-like oxidised low-density lipoprotein (LOX-1)
Urinary markers	→	Microalbuminuria; urinary albumin excretion rate (UAE)
Miscellaneous	→	Troponin T & I; pro-brain B-type natriuretic protein; glycosylated haemoglobin (HbA1c); serum creatinine; homocysteine

# Improving results of revascularization

## Percutaneous transluminal stenting

Annually nearly 4 million bypass or stenting procedures (coronary artery and peripheral arteries) are performed worldwide. The advantages of radiologically-placed stents in both coronary and peripheral arteries are numerous. While the advances in drug-eluting stent technology allow intervention in more distal vessels, there is still a substantial thrombosis rate associated with inhibition of smooth muscle and endothelial cells. This stent platform has recently been used to deliver protective endothelial nitric oxide synthase (eNOS) gene therapy locally. In normal and hypercholesterolaemic animal models, these gene-eluting stents enhanced endothelial regeneration while reducing neo-intimal formation when compared to controls. This platform could be used to deliver other or multiple genes and combinations of agents if necessary.

## Small-diameter vascular grafts

There will always be patients who require bypass grafting and the 'gold standard' grafts are autologous vessels (mainly venous but also arterial). However, no suitable vessel is present in >40% of patients and currently synthetic (eg. Dacron) grafts are used. Synthetic grafts have higher failure rates when compared to autologous conduits, for many reasons, including thrombogenicity of the graft surface and the formation of intimal hyperplasia resulting from a compliance mismatch between the graft and the native blood vessel. These failure rates are particularly high when small-diameter grafts (<6 mm) are used in coronary, lower limb and plastic reconstructive operations. Thus there is a need for the development of a biomechanically stable and biocompatible small-diameter vascular graft. Other vascular conduits have been used, including allografts and xenografts, but these have limited 5-year patency rates because of immune-mediated rejection. In addition, the ethical issues and the risk of infection transmission surrounding xenografts have limited their widespread use.

This problem is being addressed here in Leeds using a tissue-engineering approach, which offers the possibility of developing biological substitute material using natural scaffolds. Using this technique to develop a small-vessel graft poses many challenges, including recapitulating the physical and biological properties of the natural tissue, preventing host rejection and adequate remodelling over time. The 'Leeds Principle' involves decellularizing native scaffolds and implanting these *in vivo*, either as such, or following recellularization with endothelial and smooth muscle cells (Figure 1). Once combined in the correct manner, it has been proposed that a dynamic and interactive cell phenotype and extracellular matrix could be produced, which would be able to grow, develop, repair and remodel itself similar to native tissue. The clear advantage of this strategy is that xenogeneic scaffolds could offer an unlimited source of vascular grafts. The challenge of this approach is the achievement of complete decellularization, removing all immunogenic debris, whilst maintaining the mechanical and structural integrity of the matrix.

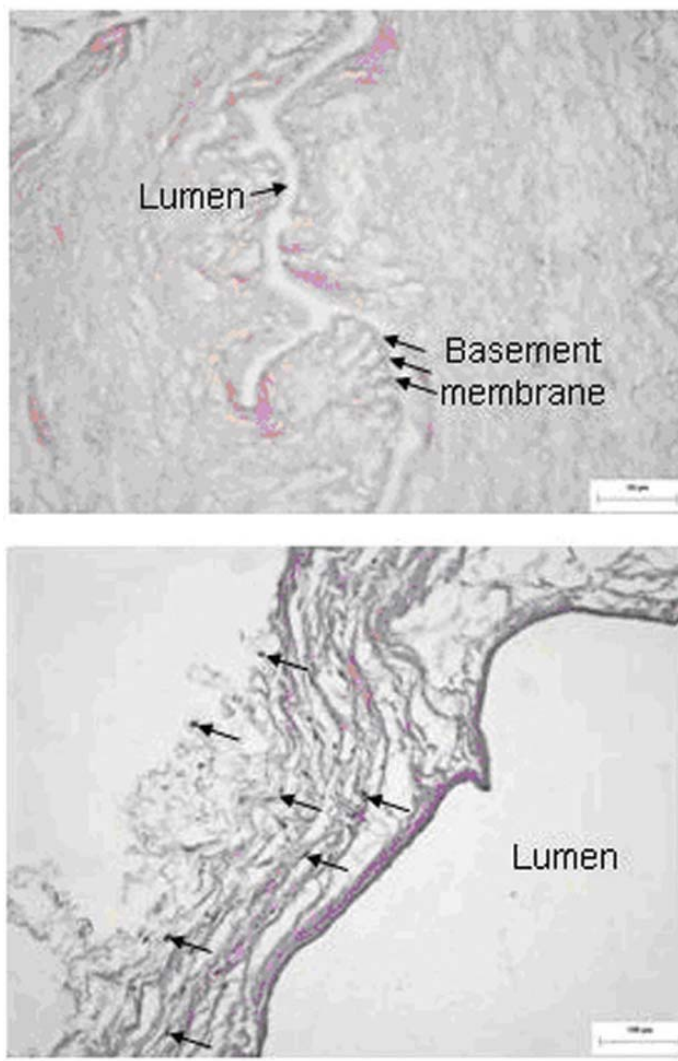


Figure 1. Recellularization of an extracellular matrix. (A) A bovine ureter has been decellularized using a novel protocol to leave a collagen matrix. (B) Isolated and cultured vascular smooth muscle cells have been incubated with this scaffold for 7 days and have attached to the matrix (indicated by arrows). Images courtesy of T Khan (Institute of Medical and Biological Engineering, University of Leeds, UK).

## Founding partners in the Programme include:

University of Leeds  
University of Sheffield  
University of York  
University of Bradford  
Medipex  
Medilink Yorkshire & the Humber  
The Leeds Teaching Hospitals NHS Trust  
Sheffield Teaching Hospitals NHS Foundation Trust  
Bradford Teaching Hospitals NHS Foundation Trust  
Yorkshire Forward  
Health Technologies Knowledge Transfer Network  
New Jersey Biotechnology Life Science Coalition  
Rutgers, The State University of New Jersey  
University of Medicine and Dentistry of New Jersey  
New Jersey Institute of Technology  
Princeton University  
International ARI Institute, University of Toledo, Ohio  
Polymer Centre for Industrial Collaboration  
Biomaterials and Tissue Engineering Centre for Industrial Collaboration  
Pharmaceutical Innovation Centre for Industrial Collaboration  
Wireless Technologies Centre for Industrial Collaboration  
Particles Centre for Industrial Collaboration

*“Medicine will change more in the next twenty years than it has in the past two thousand.” L Turnberg (1997)*

The speed at which new and emerging technologies are being developed is staggering. There is a real feeling that these technological advances hold the key to major improvements in healthcare. This sentiment is reflected in the areas outlined in this short report. However, it is crucially important that clinical and patient input forms part of the collaboration between science and industry, to optimize the care of patients.

## Selected Centres of Excellence

### Imaging

Molecular Imaging Program at Stanford University ([mips.stanford.edu/](http://mips.stanford.edu/))

Center for Molecular Imaging Research at Harvard University ([cmir.mgh.harvard.edu/](http://cmir.mgh.harvard.edu/))

### Biomarkers

Cardiovascular Research Programme at Harvard University

([www.brighamandwomens.org/research/Cardiovascular/default.asp](http://www.brighamandwomens.org/research/Cardiovascular/default.asp))

The School of Chemistry, Leeds University

([www.chem.leeds.ac.uk/People/Fisher.html](http://www.chem.leeds.ac.uk/People/Fisher.html))

### Tissue engineering

Institute of Medical and Biological Engineering, Leeds University

([www.imbe.org.uk/](http://www.imbe.org.uk/))

Cytograft Tissue Engineering

([www.cytograft.com/](http://www.cytograft.com/))



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