

Near patient testing: diagnosing a first world killer

Ana Gallardo-Soto, Keith Rawson and Calum McNeil describe how research in the biotech sector is spearheading the search for a novel approach to the diagnosis of one of the western world's biggest killers – myocardial infarction

DESPITE the steady decrease in the number of deaths since the 1970s, heart and circulatory diseases continue to be the main killers in Western societies with 236,473 adults losing their lives to cardiovascular injury in 2000 in the UK only (153,134 patients died of cancer in the same year). According to the British Heart Foundation, coronary heart disease (heart attack and angina) alone was the cause of more than 20% of the deaths in the UK in 2000. Modern living habits and poor diagnostic efficiency increase the spread of this new epidemic, which is currently costing the NHS approximately \$15m every year in prevention, detection and treatment.

Acute myocardial infarction (AMI) is the most common and recurrent final expression of cardiovascular tissue damage, being responsible for almost 700,000 deaths in the US last year. It is generated by a sudden reduction in coronary blood supply causing heart muscle (myocardial cell) death in the affected area. Due to the immediate consequences of

heart failure, rapid and appropriate diagnosis or exclusion of AMI is crucial for the successful treatment of patients presenting to casualty departments with chest pain. Unfortunately, the performance of current diagnostic tools is far from ideal: not only do more than 30% of heart attack victims die before reaching the hospital in the UK, but prompt and accurate diagnosis of AMI still remains a major issue in hospital cardiology units. In a pilot study of patients presenting with

chest pain at the accident and emergency department of the Royal Infirmary in Newcastle (UK), AMI was wrongly diagnosed in 35% of the cases and 5–10% of patients' AMIs were missed, despite application of conventional diagnostic strategies. Moreover, in only 60% of the cases was AMI diagnosis confirmed within the first 12 hours of onset of the symptoms.

Cambridge Life Sciences (CLS) together with the medical school of the University of Newcastle-upon-Tyne has addressed this overwhelming problem by developing a quantitative near-patient immunosensor technology to be used for myocardial infarction diagnosis and cardiac monitoring. The novel device enables rapid and quantitative prediction of infarction episodes as well as reliable cardiac monitoring by specialists in emergency units, paramedics, GPs, and other professionals.

Missed myocardial infarctions are indeed the leading cause of malpractice in emergency medicine in the US with up to 50,000 AMI patients sent home inappropriately each year. AMI early diagnosis would permit rapid admission to coronary care and intensive care units (CCU and ITU respectively), as well as avoid premature discharge of patients from the accident and emergency department, reducing drastically the number of casualties for incorrectly ruled out infarctions. On top of this, avoiding unnecessary CCU/ITU admission of patients without AMI would also bring important economic and human advantages, saving up to 50% of bed occupancy in coronary care units and allowing outpatient management of low infarction risk cases.

Criteria for diagnosis of AMI are most commonly based on the patient's clinical history, examination by the physician and electrocardiogram tests. Although helpful in many cases, such techniques are imperfect for accurate diagnosis and profoundly depend on correct subjective interpretation. Hence patients are sent home prematurely or AMI is incorrectly

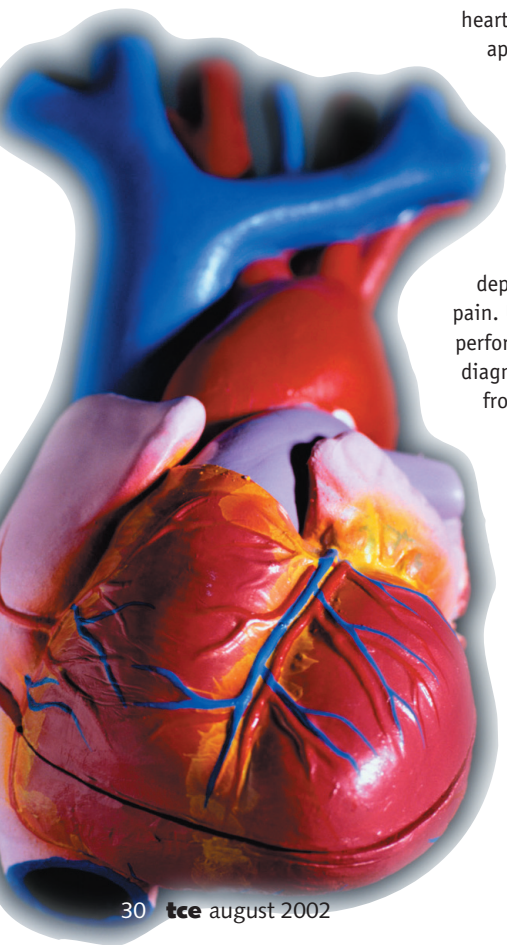
diagnosed, with the resulting inappropriate use of hospital resources.

Crushing chest pain, radiating to the shoulders, arms or abdomen and persisting for more than 20 minutes is usually the initial symptom of an infarction, and electrocardiograms (ECG) are commonly the first tests to perform to patients suspected of AMI when reaching the accident and emergency department. Despite its high specificity, ECG is a poorly sensitive triage tool, giving inconclusive and unreliable results in approximately 50% of the suspected cases of infarction. It is when ECG findings are equivocal that the measurement of the concentration of certain cardiac enzymes in the blood stream appears most useful. These so called cardiac markers are proteins normally contained within the myocardial cells' cytoplasm, released into the blood circulation when damaged.

As an example of the current diagnostic procedures followed in suspected AMI cases, according to a US study from 1997, five out of eight million patients reaching emergency units with chest pain were admitted to hospital to rule in or rule out AMI. One million of these did in fact develop an infarction in the following hours: 500,000 of the infarction cases were picked up by standard ECG and the rest were later ruled in using ECG and cardiac markers.

In order to facilitate early diagnosis and therefore improve AMI management substantially, a useful marker of myocardial damage should present the following characteristics:

- rapid release, so that the test becomes diagnostic early after the onset of infarction symptoms;
 - easy to assay, preferably with a very rapid turn-over time;
 - heart specific – that is, its concentration should not increase significantly as a consequence of other cardiac clinical conditions;
 - supported by reliable clinical research with appropriately designed studies in the population in to which the test is intended to be applied.
- Currently available cardiac markers fall



far from fulfilling such requirements. Creatine Kinase MB isoenzyme is relatively myocardial specific but lacks sensitivity due to its slow release (its concentration does not reach diagnostic performance until four to ten hours from onset of infarction). Thanks to its small size, myoglobin is rapidly released into the blood circulation and also rapidly cleared by the kidneys, which makes it highly sensitive at early stages and interesting for re-infarction detection (its levels rise in two to three hours and return to normal within 24 hours). However, its abundance in skeletal muscle makes it unspecific. Concentrations of troponin T and I, structural proteins specific to cardiac muscle, may take up to 12 hours to become elevated after an episode of myocardial damage and remain so for several days, thus making the detection of recurrent episodes of AMI impossible.

In a recent multi-centre study (EUROCARDI) carried out jointly by the medical school of the University of Newcastle (UK), the University of Maastricht (Netherlands) and research teams from Denmark and Germany, fatty acid binding protein (FABP) was pointed as the most sensitive biochemical marker for early diagnosis of AMI. FABP is a small (15 kDa) cytoplasmic protein abundant in heart muscle cells. The fact that it shows a ratio of cytoplasmic to vascular concentration one order of magnitude higher than any other cardiac protein makes FABP the most sensitive and specific marker for AMI diagnosis within three hours of the appearance of chest pain symptoms. Not only does it present 100% sensitivity, it is also 29% more sensitive than myoglobin and 35% more sensitive than CK-MB in such a time window.

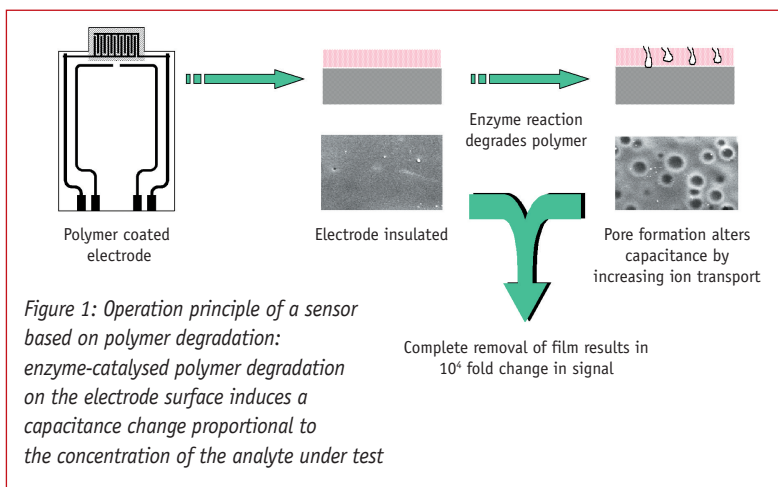
Plasma levels of FABP return to normal within 12–24 hours, which is important for picking up recurrent AMI incidents. Reperfusion after administration of thrombolytic therapy is usually monitored by electrocardiographic criteria, despite the low reliability of ECG in AMI diagnosis. Myoglobin levels are also measured to assess the probability of unobstruction of the infarct related artery (IRA) at appropriate times. Since FABP is detectable in peripheral blood earlier than myoglobin and has significantly lower background levels, it should prove to be a more sensitive predictor of the degree of vascular blockage than myoglobin. With highly invasive interventional procedures

increasingly being used in patients with assumed failed reperfusion, accurate and early prediction of IRA patency with a simple blood test is a definite and profound step forward.

The combination of what we believe to be the best early marker of myocardial damage with a transducer system allowing rapid, accurate measurement of this marker is ideal for its application in point-of-care or near-patient testing. Such a selected transducer emerges as the result of putting together the specificity of immunoassays with the high sensitivity of AC impedance detection techniques.

AC impedance enzyme electrodes measure the change in the impedance, admittance, or any of their components (capacitance, conductance, susceptance or resistance) in a sample arising from a chemical reaction. AC impedance biosensors can be applied to any biological or chemical process that implies a variation in the electrostatic characteristics of the sample under test. Therefore, generation and consumption of ions, migration of charges, changes in the dipolar profiles and so on can be monitored by AC impedance methods. Their broad applicability, high sensitivity and relative simplicity of use have promoted extensive study in different fields, ranging from immunosensors to microelectronic enzyme electrodes. Changes in the characteristic impedance at a particular frequency, typically in the kHz range, which take place as a result of a biological/chemical reaction in the tested sample, are recorded and correlated to the substrate concentration.

The immunosensor device developed jointly by CLS and the medical school of the University of Newcastle is based on capacitance measurements of enzyme-catalysed polymer degradation. It works by monitoring the capacitance changes in an electrode, which result from the partial or complete removal of an insulating polymer film from its surface due to the occurrence of an enzymic reaction. Considering a film of



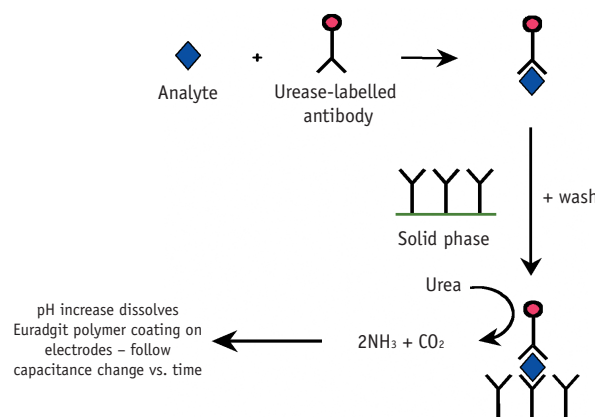
approximately 1 μm, complete removal of the polymer coating increases the capacitance by four orders of magnitude, therefore as few as 10¹² molecules can cause a significant change in the electrode impedance. Figure 1 shows a diagram of the operation principles of the sensor.

Electrodes are coated with polymers designed to dissolve at a regulated alkaline pH. These materials are already in common use in the pharmaceutical industry in the form of enteric coatings, which allow orally delivered drugs to dissolve in specific areas of the alkaline intestine, avoiding the acid conditions of the stomach and providing a targeted drug delivery. Such polymers can be tailored chemically to control their properties and form uniform and controllable films with no defects, which can then be reproducibly screen printed onto the electrode surface. The Röhm Pharma enteric polymer Eudragit S100, which degrades rapidly above pH 7.4, was chosen.

The FABP detection principle behind the sensor is based on a two-stage antibody capture sandwich-type enzyme immunoassay – see Figure 2.

The test involves a nitrocellulose

Figure 2: FABP detection principle: two stage antibody capture sandwich-type enzyme immunoassay



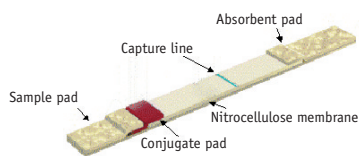


Figure 3: Typical lateral flow test strip

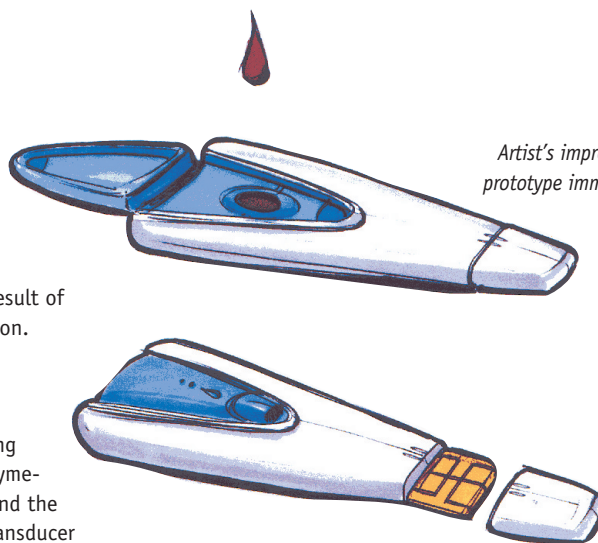
lateral flow strip on which FABP monoclonal antibody (MAb) has been previously deposited as a test line, and urease-conjugated FABP MAb acts as a mobile phase – see Figure 3. The soluble antigen (FABP) present in the blood or plasma sample combines with the first specific antibody, which is chemically conjugated to active urease. The bound FABP, now linked to urease, is captured by a second anti-FABP antibody, this time bound to the surface of the flow strip material.

All residues of unbound antibody-urease are then eliminated introducing a wash solution containing urea, which is rapidly hydrolysed in the presence of urease, releasing carbon dioxide and ammonia and increasing the local pH to values above eight. The polymer film starts degrading under these alkaline conditions, altering the insulating electrode coating and causing a measurable change in capacitance. Such a capacitance variation is directly related to the analyte concentration present in the sample, giving a quantifiable reading of FABP concentration in less than ten minutes. Figure 4 shows typical capacitance curves recorded with the immunosensor in whole blood samples. The concentration of FABP had been previously calculated through standard ELISA methods.

Not only is the immunosensor presented here able to clearly differentiate the concentration of FABP

in a reproducible manner, but the detection system also offers the advantage of being easily applicable to the measurement of many other biomolecules produced as a result of a disease or abnormal condition. Indeed, the combination of a lateral flow strip device with polymer breakdown allows generic applicability by varying the mobile antibody, the enzyme-conjugated bound antibody and the antigen chosen. As for the transducer role, impedance measurements are intrinsically very sensitive, which makes them an appropriate option for the detection of multiple analytes.

In fact, we are currently investigating the performance of such a sensor in the diagnosis of prostate cancer though the measurement of prostatic specific antigen (PSA). PSA is a single-chain glycoprotein produced by the prostate gland and mainly present in its epithelial cells. Although most of the PSA is eliminated from the body in semen, a very small amount escapes into the blood stream. It is this amount that is employed as a common indicator of the occurrence of prostate carcinoma. PSA can appear by itself as free PSA or bound to other substances in the blood stream. Since the concentration of total PSA in blood is normally extremely low (4 ng/ml is considered the threshold value for the diagnosis of prostate cancer) any PSA test requires a very sensitive detection method based on monoclonal antibody technology. The proven high sensitivity and specificity of the prototype immunosensor makes it ideal for the measurement of PSA in whole blood or serum. Not only does it achieve easily the requirements for such a test but it also goes a step forward, introducing



Artist's impression of a prototype immunosensor device

demonstrate the feasibility of development of such a simple impedimetric immunosensor device, which allows the detection of both total and free PSA in less than ten minutes at the concentration levels required for the screening of prostate cancer in men.

Therefore oncology, as well as other fields such as brain damage, could benefit from the advantages of the generic immunosensor operational principles, making the current FABP sensor the first one of a family of near-patient diagnostic devices allowing fast quantitative analysis on a reliable basis. Such a powerful assessment tool could also make use of the vast potential of telecommunication services, increasing extraordinarily the number of possibilities for its use. Not only would it be suitable for standard laboratory procedures, but tests could be performed by GPs in routine check-ups and by paramedics in ambulances or emergency situations outside the hospital. Providing an appropriate communication network, the results could be transmitted directly to the clinic enabling immediate treatment of the patient on arrival. Its simplicity of use could even make it a user-friendly device to be employed by chronic patients at home; these again could be linked by a simple telephone line to the GP surgery or hospital, where relevant steps should be taken if necessary.

In any case, the potential impact of this family of diagnostic devices under development is enormous. Their application to myocardial infarction detection is considerable, particularly if it leads doctors to more accurate and earlier AMI diagnosis, allowing better treatment and detection of higher risk patients and better monitoring and quality of life to those chronically suffering from this first world killer. ■

Ana Gallardo-Soto, Keith Rawson and Calum McNeil work for Cambridge Life Sciences

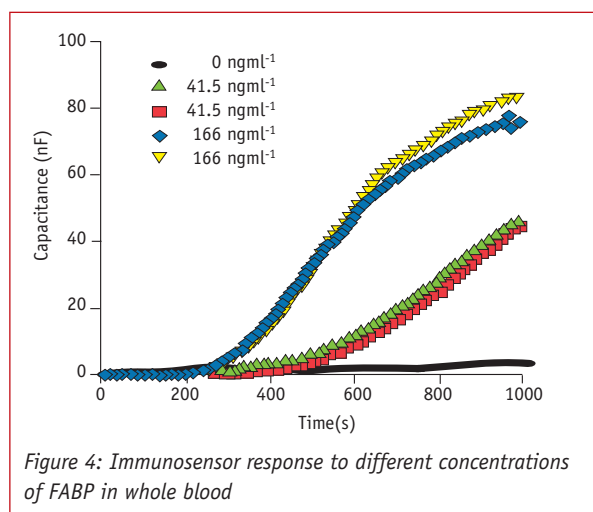


Figure 4: Immunosensor response to different concentrations of FABP in whole blood