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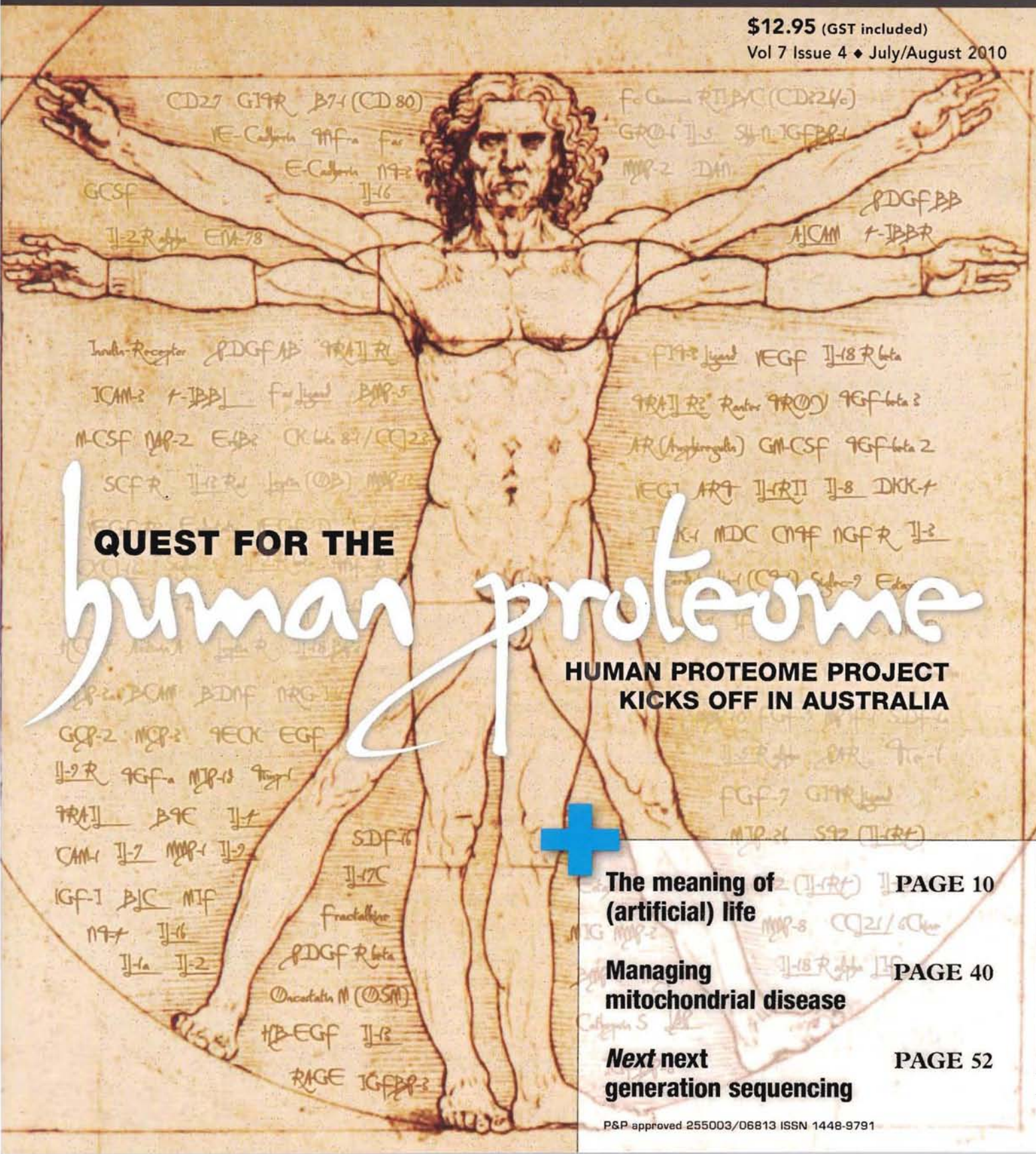

QUEST FOR THE human proteome

HUMAN PROTEOME PROJECT KICKS OFF IN AUSTRALIA

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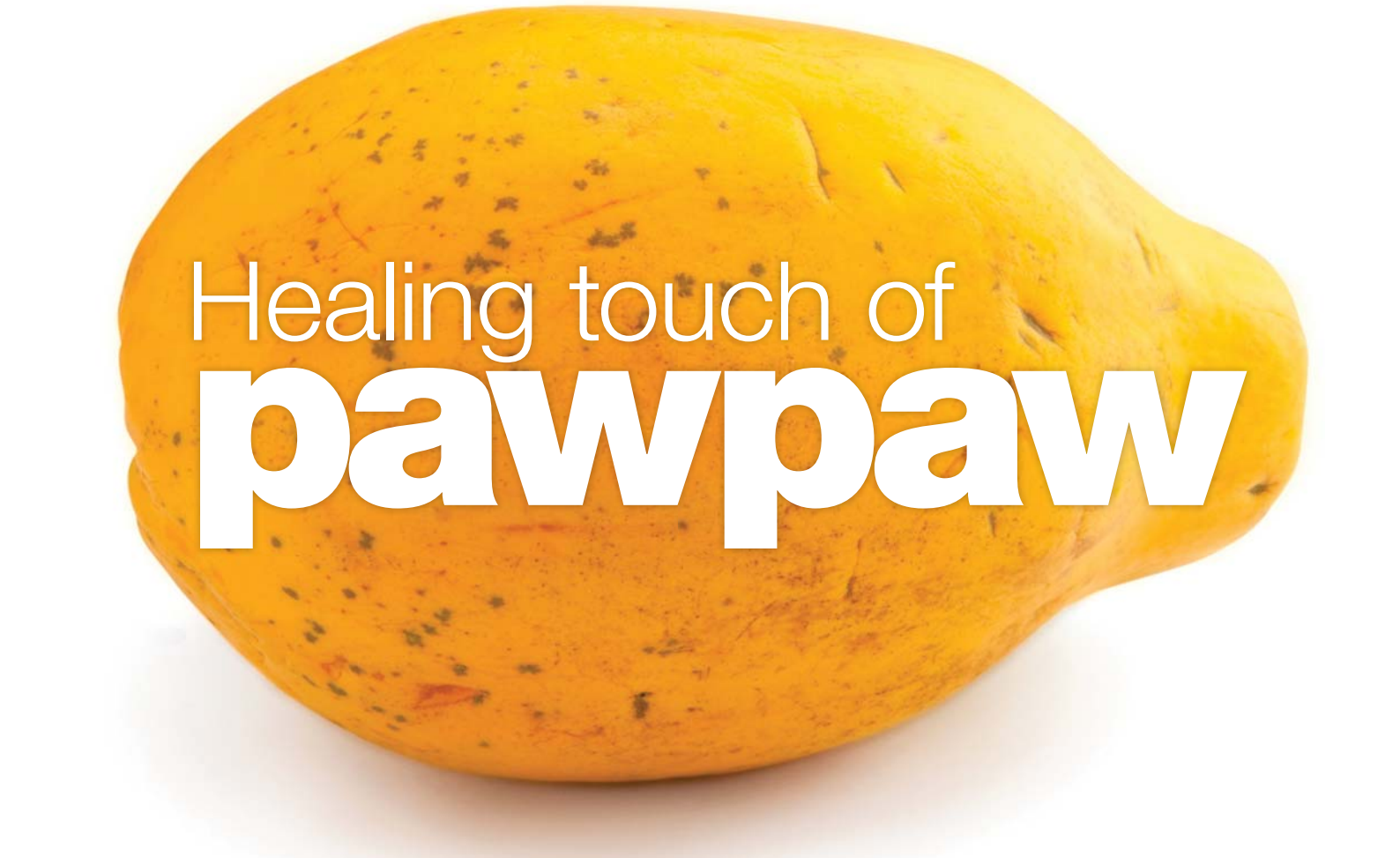

Healing touch of pawpaw

The first clinical trial of an Australian-made wound healing product derived from pawpaw, *Carica papaya*, is underway in Melbourne. While the exact modes of action are still unclear, proteomic and enzymatic approaches are being explored to understand how the extract got its power.

By Kate McDonald

IT MAY SOUND LIKE THE SPIEL of a snake-oil salesman, but the story behind the development of a new Australian product derived from an extract of pawpaw (*Carica papaya*) designed to promote wound healing is a compelling one. The Australian company behind it, Phoenix Eagle, has developed a new product, OPAL A, that could revolutionise wound healing and wound care.

The story is an unusual one to say the least, but in the company's favour is the quality of the people it has managed to get on board. Not only does it have local and international specialists as advisors, but it has managed to recruit one of Australia's best known wound management experts, Associate Professor Michael Woodward of the University of Melbourne's School of Medicine, and past-president of the Australian Wound Management Association (AWMA), to run its early phase clinical trial at the Heidelberg Repat, based at the Austin Hospital.

A clinician as well as a researcher, Michael Woodward admits to being a bit sceptical about the story when he first heard it. "Whenever anybody says a vegetable extract does miraculous things, the first reaction of a doctor is to be a little bit doubtful," he says. "That's not because we are all in the pockets of the pharmaceutical industry and only believe in expensive drugs, it's just because many claims have been made about foods and vitamins in the past that have not been proven once they've been subjected to the light of evidence."

But, having witnessed some remarkable results after the product was used to treat pressure ulcers at Perth's Quadriplegic Centre, he has come on board. Woodward is now looking for evidence that OPAL A does indeed promote wound healing, running a blinded, randomised placebo-controlled trial of the product in people with hard-to-heal venous and pressure ulcers. The candidates will receive either a placebo or a treatment with OPAL A filtrate, applied directly to

the wound, as well as a 30 per cent-strength cream applied to the surrounding area.

Only those who are not responding to regular therapy will be recruited, Woodward says. "We don't want a new product that is no better than what we have already. For that reason we have everybody treated for four weeks with regular therapy and then at the end of that four-week period they only get into the study proper if their ulcer hasn't healed by 25 per cent of the surface area," he says.

"The good thing, if it turns out to be effective, is that it is a totally Australian invention that most people will feel comfortable with," Woodward says. "A lot of people are more comfortable putting something that is taken from pawpaw on their wound than something that has been developed in a test tube with genetic modification."

PATENTED PROCESS

The story behind the product's discovery is a compelling, if slightly unusual. The inventor, Tom McArthur, has no scientific training but has been responsible for a number of inventions. According to Phoenix Eagle's managing director, Mark Richardson, McArthur has been interested in the therapeutic properties of fruits and vegetables for many years. This was rekindled when he was in the British army based in Malaya in the 1950s.

He spent many years experimenting and finally came up with a way to extract a filtrate from the pulp of the fruit by heating it and adding sodium bicarbonate. Many different fruits and vegetables can be used in this process, but pawpaw looked the most promising. The company has taken out a global patent application on the process, the products derived from the process and the uses of these products, and has filed this application in the major global markets.

It was the cosmetic applications that first sparked McArthur's interest, but the more important application, for the healing of chronic wounds, came about by chance, says Richardson. McArthur began to treat an acquaintance who had an ulcerous toe that had become necrotic and had been scheduled for amputation, having already lost three others. The treatment, which was videoed, was successful and the patient kept his toe. Another patient, confined to a wheelchair and suffering from a large ulcer on his hip and a hard scar on his buttock, was also successfully treated. Then, Richardson received a phone call from the director of nursing at the Quadriplegic Centre in WA, where the product was unexpectedly being trialled by one of the nursing staff, with excellent results.

That's when things started to look promising, Richardson says. The idea was initially to manufacture the product for cosmetic uses, but its excellent – albeit anecdotal – results in chronic wounds showed that it should be taken more seriously. So Professor Geoff Mitchell, a general practitioner, palliative care specialist and professor of medicine at the University of Queensland in Ipswich, was asked to take a look.

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"The inventor treated patients on an informal basis and then they started keeping records, which included taking photographs of the treatment process as time went on," Mitchell says. "They asked me to be an independent observer and to correlate what these people had observed with medical records, hospital records and nursing service records so that we could have a credible story that would be accepted by the profession."

Mitchell presented five cases, which he was not involved in, at the AWMA conference in Darwin two years ago, then at the recent AWMA 2010 conference in Perth, where he presented the results of seven more, six of whom he had treated personally. The wounds ranged from pressure and venous ulcers, to skin tears and an infected traumatic ulcer in a man who was chronically hypoxic from chronic obstructive pulmonary disease.

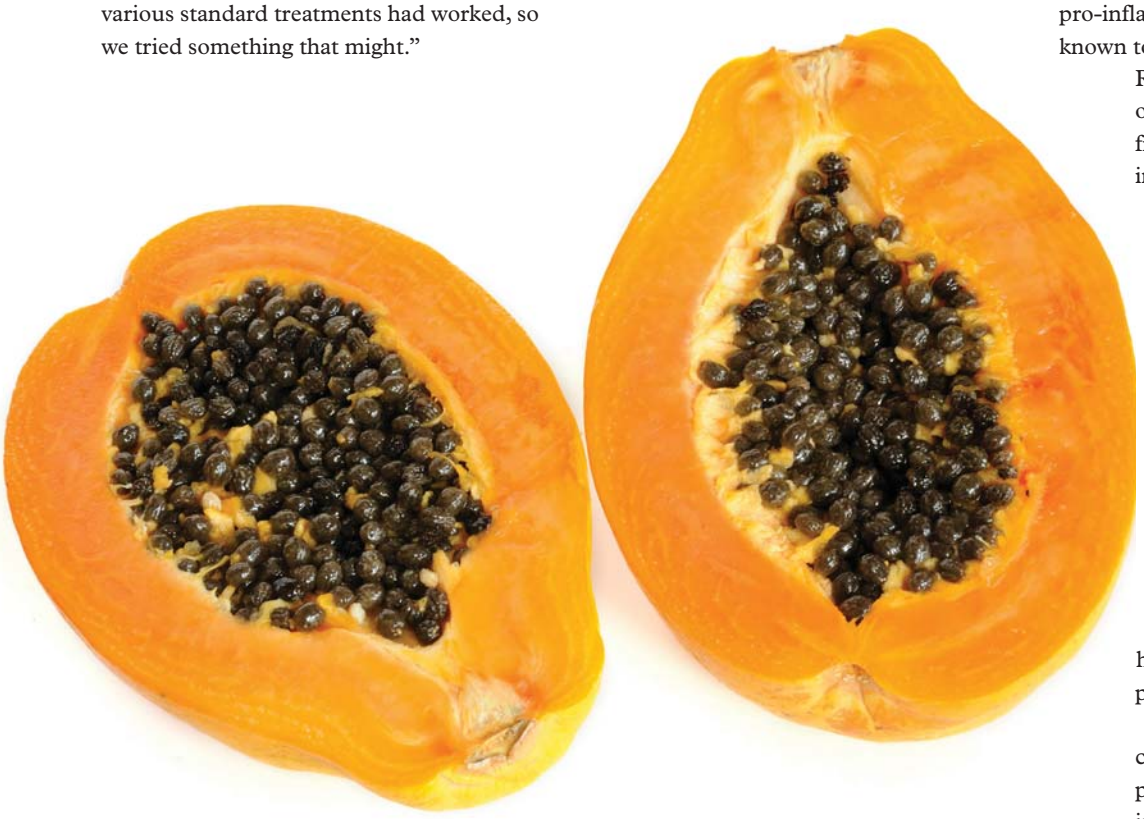
"They were all chronically unwell patients but with different causes of their disease," Mitchell says. "The product has only been used on people where normal treatments have been trialled and failed. We were at the point where the patients had had the best that is on offer and none of the various standard treatments had worked, so we tried something that might."

The results were excellent, with significant wound healing – properly documented and photographed – in most cases. There was one adverse event, resulting in the amputation of a toe which had appeared to be marginally viable before treatment with OPAL A had commenced.

MODES OF ACTION

Mitchell is engaged to advise Phoenix Eagle on medical issues through the University of Queensland's commercialisation company, UniQuest, although he retains his independent role. He was introduced by the company to Dr Fraser Russell, a biochemical pharmacologist from the University of the Sunshine Coast, who is investigating the possible modes of action of the filtrate.

While the scientific explanation of the therapeutic properties is as yet poorly understood, there are several possible modes of action, Russell says. One he is investigating is the product's anti-oxidant properties, another is the inhibition of a pro-inflammatory pathway involving the 5-lipoxygenase enzyme.



"The antioxidant potential of OPAL A was assessed by measurement of its free radical scavenging activity," he says. "Full retention of scavenging activity was observed with samples of OPAL A that were a hundred-fold less concentrated than that used in the clinical trial, suggesting a very good antioxidant response."

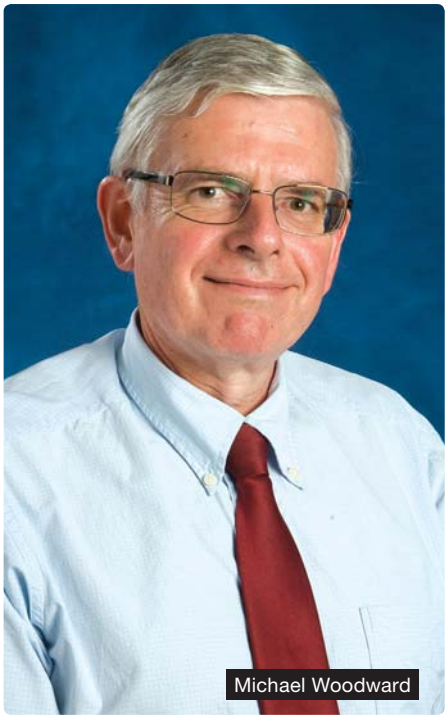
Using a cell-free assay, OPAL A was also found to inhibit 5-lipoxygenase, an enzyme responsible for catalysing the production of the pro-inflammatory mediator leukotriene B4.

One of Mitchell's observations in treating patients was that OPAL A tended to improve perfusion of the wound site with blood, suggesting a vasodilator response. This hypothesis has been supported by Russell, who has shown that OPAL A relaxed isolated segments of pre-constricted human blood vessel. The relaxant response was attenuated by an inhibitor of nitric oxide synthase, suggesting that OPAL A mediates this response through production of the endothelial vasodilator substance, nitric oxide.

"We hypothesised that OPAL A might facilitate wound healing by tempering pro-inflammatory responses that are known to occur within the wound region," Russell says. "In support of this, our early findings using cell-free assays showed that OPAL A inhibited purified 5-lipoxygenase, an enzyme responsible for hastened production of the pro-inflammatory mediator leukotriene B4.

"In addition, OPAL A was also found to have antioxidant properties. Our current studies are aimed at extending these observations by examining the ability of OPAL A to modulate the production of reactive oxygen species and leukotriene B4 in neutrophils. This work follows our recent studies that have identified vasorelaxant properties of OPAL A.

"The relaxation of a pre-contracted human blood vessel preparation was sensitive to an inhibitor of nitric oxide synthase,


Michael Woodward

indicating an endothelium-dependent, nitric oxide-mediated response. The findings raise the possibility that OPAL A might improve supply of oxygen and nutrients to tissue in the wound site and thereby facilitate wound healing."

ENZYMATIC AND PROTEOMIC APPROACHES

In trying to elucidate the modes of action of the compound, Phoenix Eagle commissioned a search for the leading global researcher on the enzymes of *Carica papaya* and came up with Keith Brocklehurst, Emeritus Professor of Biochemistry at the School of Biological and Chemical Sciences at Queen Mary, University of London, who has 40 years' experience of conducting mechanistic studies on the enzyme components of *Carica papaya*. Brocklehurst has conducted a review of Phoenix Eagle's early work and has indicated his interest in overseeing one of the planned programs of studies to be undertaken.

"A barrier to understanding the mechanism of the therapeutic functions of OPAL A is that it is not known which components of the papaya fruit contribute and in what ways," Brocklehurst says. He is planning a collaborative program of research involving protein chemistry and enzymology, directed by himself, and biological assays of tissue repair for phenomena such as cell proliferation, viability and migration, directed by Mike Philpott, professor of cutaneous biology in the School of Medicine and Dentistry at Queen Mary.

Phoenix Eagle is also using a number of proteomic approaches to characterising the molecular structure of the compound, working with one of Australia's pioneers of the application of mass spectrometry-based protein and peptide drug discovery, Dr Richard Lipscombe, managing director of Perth-based specialist clinical research organisation Proteomics International and an adjunct professor at Murdoch University.

Lipscombe and his team are using liquid chromatography-matrix assisted laser desorption ionisation mass spec (LC-MALDI-MS) to improve protein separation and sensitivity and to enable high-throughput time of flight (TOF) instruments to process the samples. "It also means the samples are stable on the target so it can be archived and processed at a convenient time, and the samples can be re-analysed," Lipscombe says.

"When applied to OPAL A the findings were surprising. In contrast to the untreated papaya extract, which showed most proteins were degraded beyond recognition, the OPAL A processing transformed the protein profile. This time the bioinformatics analysis detected a suite of new proteins, all with putative biological activity. The exciting next step will be to investigate these molecules in more detail and seek to understand how the OPAL process produces them and what their bioactive role could be."

While the proteomics research is underway, all eyes are on the proof of concept trial at the Austin. While there will be doubters, Woodward reminds the sceptics that some of the best-used drugs in the world are naturally derived, including Digoxin, a treatment for heart disease that is derived from digitalis; the dementia treatment Galantamine, which is derived from Russian snowdrops; and the new cancer drug Paclitaxel (marketed as Taxol), which comes from the Pacific yew tree. "I don't think doctors are automatically sceptical of a drug that comes from a natural source, but they do want the science to show that it works," says Woodward. ALS