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DEVELOPMENT OF NOVADAQ SPY™ CARDIAC IMAGING INVENTION

I was interested in what happened to the heart when blood supply was stopped (e.g. heart attack due to vessel blockage, or stopping flow for surgery) and then restarted again (e.g. angioplasty or restoration of flow at surgery). I was mainly using magnetic resonance imaging and spectroscopy techniques to investigate what was happening to the heart muscle during this ischemic period and at reperfusion. I was also interested in ways to look at what was happening to the blood vessels during this insult. It was well known that during ischemia-reperfusion (I-R) there can be extensive edema. I wanted to know if this was because the blood vessel walls were becoming more leaky i.e. I wanted a method to asses vascular permeability during I-R. In the course of discussions with other scientists at IBD it was mentioned that indocyanine green bound extensively to plasma proteins and fluoresced with the excitation and emission maxima being in the near infrared part of the spectrum. We thought that this might be a good marker for vascular permeability as under normal conditions the ICG would stay within the blood vessels and, as it was bound to proteins, only leak out if there was significant damage to the blood vessel wall. We decided to investigate this in our ischemia-reperfusion model in isolated rat hearts. This seemed like an ideal project for a graduate student so we put the project on hold until a suitable candidate was identified.

In 1997 Rick Mangat started as a graduate student in pharmacology at the University of Manitoba. Before starting on their thesis work in this program graduate students must rotate through a couple of labs. Rick came to work in mine (I had an adjunct professorship in the pharmacology department) as part of a rotation and at the end of this rotation decided to undertake the ICG imaging project for his PhD thesis. We spent a considerable amount of time trying to obtain fluorescence images of ICG. During this time we were collaborating with a group of scientists and engineers from the Spectroscopy group at IBD – these people were experts in optical technologies including spectroscopy and fluorescence. From the literature (mainly Bob Flower's publications) we learned that maximal ICG fluorescence was observed at concentrations in the range 10-30 µg/ml. We therefore reasoned that if we wanted to see ICG fluorescence in the blood vessels with a gradual shift out of the vessels as damage occurs during I-R we should infuse ICG at this concentration over a prolonged period and observe the accumulation in the extravascular compartment. Rather than use animals in all of these early experiments we used some phantoms (e.g. grapes) in addition to isolated rat hearts.

Our experimental set up included a very sensitive camera with quite long integration times (a few frames per second??) equipped with a liquid crystal tunable filter. The camera cost in the region of $40K, i.e. this was a state of the art set up as might be expected in a leading optics/fluorescence research lab. This was used in conjunction with a 50 mWatt laser. We were unable to observe any fluorescence. Rick contacted Bob Flower by phone on several occasions seeking advice. Bob even sent us one of his 2 Watt lasers and power driver but still no success. We approached the group leader of spectroscopy (Dr. Henry Mantsch) for funds to bring Bob to Winnipeg. This request was refused and the project was put on the backburner while we turned our attention to a different approach – the use of NIR spectroscopy to look at other aspects of I-R injury.

This project was a bit disappointing in that in spite of the advances that had been made in technology the data that we could acquire was no more informative than the information that Dr. Britton Chance had gleaned 20 years previously. We did not want to pursue this. We turned our attention back to the fluorescence imaging project, but still with no success even when we tried things like pre binding ICG to albumin, purifying this product by molecular sieve chromatography and infusing this into rat hearts. In desperation we approached the Biosytems

group leader, Dr. Roxanne Deslauriers (John Docherty's direct supervisor) and requested funding to bring Bob Flower to Winnipeg stating that if he could not come we would close down the project.

Bob Flower visited Winnipeg in December 1998 for a weekend of lab experiments. We acquired very promising images of coronary arteries in the rat heart. The two key changes that allowed this to happen were:

1. The delivery of ICG as a bolus of higher concentration that arrived at the field of view at the appropriate concentration by mixing with bulk perfusate.
2. Replacing our $40K camera that acquired with long integration times with an $800 camera that acquired images at video rate, i.e. 30 frames per second.

The imaging system at this point basically consisted of a 2 watt laser passing through a lens and positioned far enough back from the heart to illuminate the entire surface and a Hitachi camera with a 330 nm [ENDNOTE No. 1] bandpass filter in front of the lens. We worked on this for a short time optimizing the image quality (changing ICG dose, slight changes to filter etc) but soon realized that the ICG does not leak out of the vasculature within the time frame that we are looking at plus the background flush caused by filing of the microvasculature precluded any way of looking at vascular leakage within the spatial resolution that we were looking at. So at this point we realized that what we could acquire were angiograms – how could we use this tool in our research. At this point I was starting to collaborate with molecular biologists who were working on a series of transgenic animal models of human disease – could we look at the intact animal. At this point we started doing some studies on mice (mot transgenic models were mice) and started with the femoral artery vascular bed with the idea of looking at changes in diameter in response to various drugs in normal mice and transgenics.

As these studies were moving along a colleague who was investigating the delivery of cardioplegia in isolated blood perfused pig hearts asked if we could use our system on his model. He was using MR perfusion imaging techniques but the resolution was not quite good enough to answer his questions. We imaged some isolated pig hearts and acquired some quite outstanding angiograms. We then thought –"could this be of clinical benefit, especially to surgeons". We had Roxanne send a fax to Dr. Wilbur Keon (with whom she had collaborated on a number of scientific projects) explaining what we were doing and if he thought that it would be of benefit. Dr. Keon was one of the thought leaders in cardiac surgery in Canada (and a Senator in the upper house of the Canadian Parliament). This very busy person responded within a day to say "yes, this is very important – I will be visiting Winnipeg within the next few weeks, show me the images and we can discuss". Dr. Keon saw the images, explained that it was his practice to inject a very high dose of ICG down bypass grafts and look at the surface of the heart to see where it turned green. What we had just done was a quantum leap ahead of what he was doing (his words). He arranged for us to contact one of his junior surgeons with a view to testing this in humans when an appropriate device was ready for human use. Attention then turned to developing and validating such a device.

We (largely Rick) had a prototype device [ENDNOTE No. 2] built by a company in Toronto. It was very basic, bringing the laser and camera together in one box to make it a simple point and shoot device rather than messing about for 20 minutes independently positioning the 2 components. This device was used to acquire some data from pig hearts for Rick's thesis (the data never did make it into his thesis). A second prototype was built by the same company – basically making it more ergonomically friendly. At this time I received grant funding from a local agency to perform a proof of concept study in pigs after they had bypass graft surgery (a collaboration with a local

cardiac surgeon). The results of this study proved that we could successfully image bypass grafts in pigs. ENDNOTE No. 3

The company ENDNOTE No. 4 was formed (April 2000) and an agreement entered into with Colorado MedTec to design and build a device that could be used in humans (Fall 2000). A two day kick off session in Boulder, CO was used to define the design requirements for the device. A Medtec team was put in place – optical engineer, electrical engineer, mechanical engineer, industrial designer) and first prototype ENDNOTE No. 5 was ready for human use in January 2001. The device was again primarily bringing together the laser and camera in one unit with a mobile cart and articulated arm. The first images were terrible. We had used the same filters (830 nm bandpass) ENDNOTE No. 6 as had been used for the pig experiment. Of course the pigs were relative babies (even though they weighed 70 kg) and the arteries were sitting right on the surface of the heart with no fat present. The patients were not so young, had arteries that did not sit on the surface of the heart and there were very large deposits of fat – we were not getting enough signal. ENDNOTE No. 7 The next few months were spent trying out a series of filters (extensive input from Bob Flower, including trying out some samples of his filters) and we ended up with an 815 nm cut filter that very efficiently transmits light >815 nm so that we could now capture pretty much all of the emitted fluorescence. ENDNOTE Nos. 8 & 9 This early device made use of a VCR to capture the images (even though we started with PC and frame-grabber in the lab) but a year later we switched over to PC to increase image quality and ease of image distribution etc.

ENDNOTES:

ENDNOTE No. 1: This is a typographical error. The filter comprises a barrier bandpass filter of 830 nm, commensurate with the peak fluorescence wavelength of ICG.

ENDNOTE No. 2: The prototype device employed a Class IIIb Lasiris Magnum 810-3000-20° semiconductor laser having a nominal optical power output of 2.7 Watt at 808 nm. The laser was tuned to a wavelength of 806 nm. The delivered optical power at the illuminated field (7.5 cm x 7.5 cm; 30 cm working distance) was 2.25 Watt, for a power density of 40 mW/cm^2. The camera was a Hitachi KPM2 RN ½" CCD Monochrome Video Camera with a bandpass filter (CVA Laser Corp. F10—830.0-4-2.00 (830 ± 2 nm bandpass, 10 nm full width half maximum (FWHM)) placed between the camera and the illuminated tissue to suppress excitation light. A Cosmicar/Pentax 16 mm f 1.4 lens was used with an f-stop of F12. The laser was housed in the imaging head.

ENDNOTE No. 3: This study involved ICG imaging of bypass grafts for pig hearts in which ICG images of excellent quality were acquired. We confirmed that this method was also applicable for CABG in humans by the time of this study, at the latest. Since the invention was completed, we filed a provisional application to the United States Patent and Trademark Office on September 24, 1999. All the practical adjustments stated in this memo after this Endnote No. 3, including the selection of the filters, were conducted based on a device, etc., identical to the invention.

ENDNOTE No. 4: The company referred to is Novadaq Technologies Inc., headquarted at Toronto, CANADA.

ENDNOTE No. 5: The laser was a 2.6 Watt Class IIIb Coherent Semiconductor F-81-2600C-200-B laser having a nominal optical power output of 2.6 Watt at 808 nm. The laser was tuned to a wavelength of 806 nm and emitted 2.0 Watt, for a power density of 36 mW/cm^2 at the illuminated field. This system utilized the same bandpass filter (830 ± 2 nm bandpass, 10 nm FWHM) that was used for the pig studies. In the clinical device, the laser light is delivered to the imaging head by means of fiber optics. The only components of the illumination system now housed in the imaging head are the fiber guide and the lens for expanding the laser output from the fiber.

ENDNOTE No. 6: The example given in the patent relates to imaging of the small animal femoral bed and the camera employs a 50 mm lens to provide sufficient magnification. The 50 mm lens has a different diameter compared to the 16 mm lens used in the pig heart studies, so that lens filter used in the pig studies was not compatible in size for this study. The 830 nm bandpass filter used has 10nm FWHM, whereas the 845DF25 bandpass filter disclosed in the patent has 25 nm FWHM. However, there is no significant difference between the two filters with respect to the transmitted fluorescence signal intensity in spite of their different FWHM, because the bandpass of the 830 nm filter is closer to the peak fluorescence of ICG than the bandpass of the 845 nm filter. Therefore, both filters should perform equally well in either application.

ENDNOTE No. 7: In addition to the noted anatomic differences between the hearts of the young pigs and the hearts of the elderly human patients, the imaging camera was enclosed in a head inside a sterile drape, which precluded changing the camera's f-stop in the operating room during imaging. Notwithstanding the above, in fact, we could obtain some signal which was sufficient for the purpose of the invention. However, if we compared these to the images we obtained from the pigs, they were not as good as expected, which is why I stated, "*The first images were terrible*". That is, while the images were in fact acceptable, they were simply not as impressive as those obtained with the pigs. Thus, some practical adjustments were made in order to obtain better images.

ENDNOTE No. 8: In order to quickly improve the image quality without making extensive adjustments to the setup, we elected to use an 815 nm cut filter, instead of the 830 nm bandpass filter previously used. The 815 nm cut filter efficiently transmits light at wavelengths greater than 815 nm, while blocking substantially all excitation light. The 815 nm cut filter has about a five-fold higher transmission of the total fluorescence signal emitted by the ICG dye over the 830 nm bandpass filter, but can also transmit a certain amount of unwanted IR outside the ICG fluorescence spectra.

ENDNOTE No. 9: It is important to note that the 830 nm bandpass filter ultimately worked successfully on human grafts in clinical trials. A study was conducted at two (2) Canadian institutions, namely: the University of Ottawa Heart Institute in Ottawa, Ontario, CANADA; and the Sunnybrook and Women's Health Sciences Centre in Toronto, Ontario, CANADA. The imaging device (i.e., SPY™ cardiac imaging device, provided by Novadaq Technologies Inc., of Toronto, Ontario, CANADA) comprised a laser diode and a driver that produced light at a wavelength of 806 nm and at a maximum output of 2.7 W.

The laser output was de-collimated to provide illumination spread uniformly over a 7.5 x 7.5 cm field of view at a working distance of 30 cm, which was identical to the dimensions used for the earlier pig heart studies. Images were acquired at a rate of 30 frames / second, using a charge-coupled device (CCD) camera sensitive to near-infrared light and equipped with an optical filter for the selective transmission of light at 830 nm. The laser, optics,

camera, and filter were integrated into an imaging head supported by a mobile arm and connected to a wheeled cart, which allowed for the system to be moved close to the surgical table at a correct focal distance above the area of interest.