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Upfront

Growing a Greener Heart

A spinach scaffold helps cultivate cardiomyocytes

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Leafy greens are said to be good for the heart, but a group of investigators have given the phrase a new dimension with their study showing the use of spinach leaves as scaffolds for culturing cardiomyocytes (1). The research tackles a current cardiac bioengineering challenge: finding a biomimetic scaffold that can form vascular systems to deliver nutrients – and can also be scaled up for clinical use.

Keen to learn more about the germination and growth of the project, we speak with first author Joshua Gershlak of the Worcester Polytechnic Institute.

How did the investigation come about?

The collaboration started when a group of professors at Worcester Polytechnic Institute, the University of Wisconsin, and Arkansas State University sat down and found that there were a lot of similar issues when researching either plant or mammalian tissue. We pondered whether we could take queues from each kingdom to aid in the research of the other, leading to the idea of using plants as scaffolds for tissue engineering.

I started talking with Glenn Gaudette – primary investigator and professor of biomedical engineering at Worcester Polytechnic Institute – about the possibility of decellularizing plant tissue as a part of our new collaboration; our lab focuses on regenerating heart tissue, so the idea grew from there.

Why spinach?

To decellularize hearts, we place a needle through the aorta before flowing through solutions for the decellularization process. We wanted plant tissue reminiscent of the vascular networks seen in the human cardiovascular system, and struck on the idea of the vein system found in spinach leaves.

We performed decellularization on a wide variety of plant tissue types including the stems of parsley, sweet wormwood, and roots, but spinach has a long stem that is easily attached to a perfusion system, a good size to allow us to easily manipulate it in the lab, and it is relatively flat which is important when we were culturing the human cells on its surface.

Did you expect it to work so well?

Plant cells are very different from mammalian cells and have a very tough outer cell wall as a form of protection. So the relative ease with which the decellularization process occurred in the plants was unexpected. For a technique that works very well in mammalian tissue to also work as well and as quickly with these tougher cells is actually remarkable. Success in the next step – getting human cells to not only attach, but act normally while on this plant scaffold – was also incredible.

Cellulose, the major component of the leaf, is a widely studied biomaterial with known biocompatibility. But cellulose used in previous studies has typically been produced from bacteria and then purified; to see this more natural form of cellulose support normal human cellular function is amazing.

What do your findings mean

for cardiac bioengineering?

To take an engineered tissue from the lab to the hospital, a vascular system is needed to provide the tissue with the proper oxygen and nutrients, especially on the microscale. Right now, fabrication techniques, such as 3D printing, aren't capable of creating a proper vascular system – and that's the major limiting factor affecting the clinical translation of engineered tissue. Using the naturally occurring vasculature in a leaf gives us new avenues to explore.

When a patient has a heart attack, there is a large non-functional scar left in the heart wall. Current surgical techniques, such as bypass surgery, reintroduce blood flow into the region but do not repair the scar. By building a vascularized piece of heart tissue using decellularized leaves, we would have the material needed to replace the scarred heart wall.

What's next?

This work has been incredibly promising but one of the first points we need to address is the biocompatibility of the leaf after processing. Purified cellulose is known to be biocompatible, but as we are using cellulose straight from the ground, we need to understand what might happen if we were to implant the unprocessed leaf into a patient. Another major question is how to allow outflow from the leaf when used in the body; human bodies have both blood supply and removal – veins and arteries – but leaves have only one system. We hope to use multiple leaves to mimic both supply and removal.

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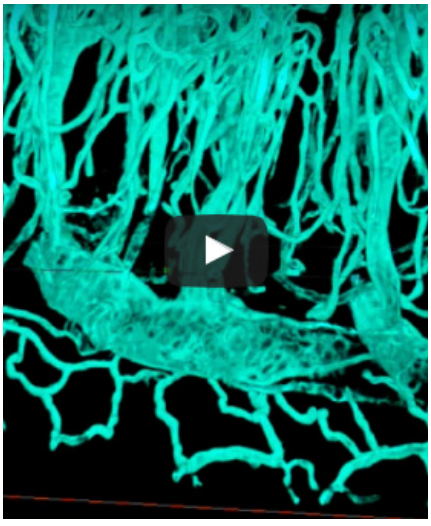
Video of the Month

We've previously discussed the vast potential of tapping into the gut microbiome and how delving into its intricacies can lead to new therapeutics (1). Now, researchers at Johns Hopkins have furthered our knowledge with their discovery of the birth- and death-cycle of gut neurons in adult mice. The work contradicts the previous belief that gut neurogenesis rarely occurs – if at all – in adult tissue (2).

The video below shows a 3D generated image of neural precursor cells (green), blood vessels (blue), and cell nuclei (grey) of a mouse gut.



Credit: Pankaj Jay Pasricha lab/Johns Hopkins Division of Gastroenterology.

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Super Sensors

A tiny biosensor could diagnose HIV within a week of infection

A Spanish team have developed an HIV test that can detect the viral capsid protein p24 at ultra-low concentrations in human plasma (1). Current HIV diagnostics are based on nucleic testing (NAT) or immunoassays. However, the sensitivity of the tests means that they can usually only detect the virus after it has been replicating for 2-4 weeks.

The new biosensor has a limit of detection of 10–5 pg/mL – equivalent to detecting one virion in 10 mL of plasma. That's five orders of magnitude better than the best immunoassay, and two orders of magnitude better than NAT, allowing detection within a week of infection. What's more, the results are ready in under five hours – a record for HIV testing.

"The prompt identification of individuals during the highly infectious acute or early stage of HIV infection has implications for both patient management and public health interventions," says Priscila Monteiro from Instituto de Microelectrónica de Madrid, Spain. Not least because the concentration of virus in plasma and genital secretions is extremely high during the first few weeks of infection.

Inside the rice grain-sized sensor, gold nanoparticles bind to the p24 protein. "Gold nanoparticles act as mass and plasmonic labels; the two signatures are detected by means of the microcantilever that serves as mechanical resonator for 'weighing' the mass of the captured nanoparticles and as an optical cavity that boosts the plasmonic signal from the nanoparticles," says Monteiro.

The team hope that the device will be particularly valuable in developing countries, which carry the highest burden of HIV. In this setting, cost is paramount.

"Right now, if we count the cost of the device (microcantilever array) and all the chemicals, the cost of the sensor is high," admits Monteiro. However, the components to construct the equipment can be fabricated en masse and at low cost, and Monteiro estimates that the device could one day be manufactured in bulk for less than 1 Euro: "Our nanosensor has the potential to become a cheap and user-friendly technology suitable for resource-limited settings in the future."

Moving the sensor into the clinic will be a long road, but the team are committed. "Getting treatment early will help people with HIV enjoy a longer life, and substantially reduce the risk of transmission to uninfected people," says Monteiro.

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Rescinding Antimicrobial Resistance

Can we make superbugs less... super?

"Klebsiella pneumoniae is probably the most threatening superbug, and a major cause of treatment failure and mortality associated with hospital infections worldwide," says Luca Guardabassi, a professor of clinical microbiology at Ross University School of Veterinary Medicine in the West Indies. The emergence of superbugs – microbes resistant to a multitude of drugs – has spurred many researchers to explore drugs with greater potency. But the team led by Guardabassi decided to tackle the problem from the other side of the fence – by reversing antimicrobial resistance (1).

To reach that ambitious goal, they used next generation sequencing (NGS) to construct a transposon library of the bacterium containing over 430,000 unique insertions, which led them to find chromosomal genes that are essential for growth during antimicrobial exposure – collectively dubbed "the secondary resistome".

How can these findings reverse antimicrobial resistance? The team proposed that inactivation of the secondary resistome using "helper drugs" (non-therapeutic compounds administered alongside drugs to boost potency) could 'restore' antibiotic susceptibility in K. pneumoniae. They've already identified a plethora of genes and metabolic pathways in the secondary resistome that have the potential for helper drug targeting, but the team is still "far away from clinical applications," according to Guardabassi. "To translate our findings into clinical practice, we need to take a further step from the antimicrobial helper drug targets that we have identified, and develop helper drugs interfering with such targets. There is still a long way to go, but I am determined to use the rest of my career to accomplish this goal."

With that goal in mind, Guardabassi and his team have set the next phase of research into motion. "We have prepared a NIH grant proposal to help us generate a catalogue of antimicrobial helper drug target candidates for all main antimicrobial classes, using the same NGS-based approach we employed in K. pneumoniae." To date, the team has identified two potential drug helpers – one anti-inflammatory and one anti-fungal – which they hope to use to generate an in

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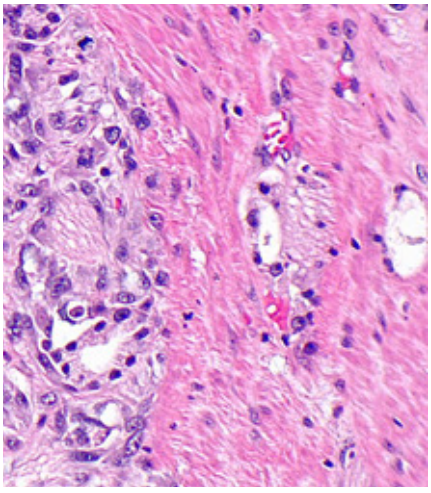

From Neurobiology to Prostate Cancer Pathology

Can the drebrin/EB3 pathway be used to predict the invasiveness of the most common cancer in men?

Metastatic prostate cancer is incurable – symptoms can only be managed – and there's currently no way to predict when or if the disease will metastasize (1). To that end, researchers at King's College London dug into the mechanisms behind prostate cancer – using knowledge gained in neurobiology – and discovered that the drebrin/EB3 pathway appears to play a role in prostate cancer's invasiveness (1). Knockdown of either protein's in vitro expression decreased the ability of the cancer cells to invade the prostrate stroma, while over-expression had the opposite effect. To find out if the pathway could be used as a therapeutic target or a biomarker for progression to metastasis, we speak with Philip Gordon-Weeks, lead investigator and professor of developmental

neurobiology at King's College London.

What do your findings mean for diagnosis and prognosis?

We haven't cured prostate cancer, but I think we've taken a big step in the right direction. A key clinical issue in prostate cancer is predicting which prostate tumors will become metastatic. Evaluating the drebrin/EB3 pathway might help clinicians stratify patients by distinguishing between benign and malignant prostate cancer. However, I don't think this would be done in isolation – one would want to examine a panel of prognosis predictive biomarkers.

The drebrin/EB3 pathway might also be a suitable target for pharmaceutical disruption. In our paper, we described using a drug (BTP2) that targets drebrin to disrupt prostate cancer cell invasion as a proof-of-principle.

How does a professor of developmental biology end up working on prostate cancer?

Well, we actually discovered the drebrin/EB3 pathway while working on the embryonic development of the nervous system. We found that it enabled embryonic neurons to respond to homing signals in the embryo that helped them to build neuronal circuits. One step in this process involves the migration of new born neurons from their birthplace in the embryonic nervous system to the final position they will occupy in the adult. The event has similarities with cancer cell invasion and metastasis – both involve homing signals and re-organization of the cytoskeleton – and so we wondered whether cancer cells might use the same cellular machinery as neurons to do this.

When we started working on prostate cancer, I thought that we would be on a one-way-street – simply applying all the conceptual insights, reagents and tools that we had worked on in our developmental neurobiology studies to investigate prostate cancer cell invasion. But we also made several unexpected discoveries about the drebrin/EB3 pathway in prostate cancer cells that encouraged us to go back and look again at developing neurons – so we were on a two-way-street after all!

What were the major challenges you had to overcome?

An experimental hurdle for us was trying to mimic the in vivo situation in a tissue culture dish so that we could more easily study cancer cell invasion. This meant setting up 3D cultures with concentration gradients of homing signals. We chose the chemokine CXCL12 as a signal because there is good experimental evidence that it is involved in stimulating prostate cancer cells to invade the prostate stroma and to metastasize to bone (2). We also wanted to test the role of the drebrin/EB3 pathway in metastasis in a pre-clinical in vivo model, but at the time there were none that mimicked bone metastasis.

What's next?

We are about to apply to the UK's Medical Research Council for support to continue our work, including exploiting a newly described pre-clinical mouse model of prostate cancer metastasis to test the role of the drebrin/EB3 pathway. These are conceptually simple experiments, but very powerful. We will edit out the drebrin/EB3 pathway in human prostate cancer cell lines using CRISPR/Cas-9, and orthotopically transplant the cells into the prostate of immunocompromised mice. If the transplanted cells multiply but fail to metastasize then this will directly demonstrate the importance of the drebrin/EB3 pathway in prostate cancer cell metastasis.

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In Perspective

Doing More with Less

Research in resource-limited settings means getting the most out of personnel, equipment and supplies – and empowering those who can continue your work

By Raffaella Ravinetto

The concept of conducting clinical trials in resource-limited settings isn't new. In fact, we've been doing it for many decades. What has changed over the last couple of decades is the nature of that work. Until about 25 years ago, most complex multi-center clinical trials were carried out in developed countries by commercial entities. Essentially, pharmaceutical companies funded their own research with the objective of bringing a new intervention to the market. But then, we started to see a shift – even the most complicated trials began to be conducted in low- and middle-income countries (LMICs). Why? Three reasons: internal validity, convenience, and neglected conditions.

Internal validity refers to the fact that new interventions must be tested in different populations. Differences in characteristics like ethnicity can change a patient's therapeutic response to a particular drug – and differences in healthcare infrastructure can impact the drug's effectiveness.

Convenience is a less pleasant concept. It refers to the possibility that an unscrupulous sponsor will conduct research in poor countries because it may lower costs, simplify ethical or regulatory review, and make recruitment easier by involving socially vulnerable populations. People with few resources often see clinical trials as a way to access free medical care – and that makes them more likely to involve themselves in research without asking too many questions about the potential risks.

The third reason, neglected conditions, deals with our growing awareness that many health problems are mainly or exclusively prevalent in LMICs, and are not yet sufficiently addressed. In the past few years, we've seen a number of positive initiatives, like new Product Development Partnerships (PDP) for research into new antimalarial treatments to compensate for the lack of efficacy of older treatments. Other new public-private partnerships conduct research into neglected tropical diseases that desperately need effective, safe and easy-to-use prevention and treatment tools. So we're really seeing clinical trials go global these days!

Shifting studies

We have always had some trials conducted in academic environments, but nowadays, more and more big trials are being carried out by noncommercial entities, which makes a significant difference to both rich and poor countries. There are some research questions that simply won't be addressed by the private sector. An example from my group's work is a comparative study prospectively comparing the safety and efficacy of existing antimalarial treatments in pregnant women. Such a study would hardly be carried out by a pharmaceutical company – after all, what if the company's product proved inferior? That's why we need independent, noncommercial research.

This kind of research also looks at fields that are less likely to turn a profit. Tropical diseases are one such area (and the reason why public-private partnerships like the Drugs for Neglected Diseases Initiative are so vital); pediatric oncology, which traditionally has low

patient numbers, is another. With some laudable exceptions, commercial research is by its nature mainly profit-driven, so whenever a research question doesn't offer a significant monetary return on investment, we rely on public funding and noncommercial entities to step up. The role they play is absolutely vital, and the more they take the lead in clinical trials, the more benefit resource-limited countries will gain.

The downside?

For science and medical professionals, the problem with noncommercial trials is that they're often under-resourced in comparison to those self-funded by pharmaceutical companies. Working in LMICs only exacerbates the problem, which often means stretching your personnel, equipment and supplies as far as they will go. More specifically, as a noncommercial sponsor, you will need to compete for external funding that is always in short supply; work in small teams where individuals may have to play multiple roles; adjust procedures according to local constraints; and maintain the highest quality, reliability and ethical compliance in your work even as you tackle these obstacles.

Let me walk you through an example – the challenge of developing new in vitro diagnostic tests for neglected tropical diseases. You would likely face a number of obstacles along the way:

Funding

To successfully compete for grants, you'll need to convince the funding agencies that your consortium is scientifically sound and can deliver quality results. At this point, you'll also need to start considering benefit-sharing – or, in other words, discovering how you can fully involve your colleagues from the LMICs in which you intend to work. How can you provide those colleagues with an equal partnership, starting from writing the grant application together? How can you ensure that your work will build their capacity to conduct independent research in the future?

Infrastructure

When you discuss working "in Africa," people often envision lifeless deserts, ramshackle buildings, starving people in rags. But in fact, our work contexts can vary widely, from state-of-the-art tertiary hospitals in large cities all the way to remote rural settings with poor medical infrastructure and no research capacity at all. You may need to begin by creating or upgrading the local infrastructure; in particular, labs that can provide routine medical care don't necessarily have the procedures in place to meet research demands. Making them suitable for research requires significant effort and investment – but if you don't do it, you can't move forward.

Recruitment

In clinical research, we consider vulnerable populations to include children, the elderly, the incapacitated, and so on. But vulnerability may be much more widespread, especially (but not exclusively) in LMICs. Often, resource-limited settings lack social security systems and accessible healthcare, which is why many patients view clinical trials as a way to obtain free treatment. It's difficult – but vital – to ensure that you're not unintentionally exploiting that vulnerability when recruiting a patient population. You must also ensure that those who aren't eligible for the trial are not treated as "second-line" patients and still receive some benefit from its presence – for

instance, by upgrading local laboratories so that everyone receives better care.

Engagement

Not every researcher is a born communicator – and dialog becomes even more complex when you have to translate into local languages. I've seen many studies regarding patients' capacity to understand research, but very few that closely examine the researchers' ability to explain it. I think we need to ensure that researchers are trained in empathetic communication (see "The Missing Piece of the Puzzle"), and what's more, I agree that we need to familiarize ourselves with local customs and cultures early on – perhaps with the aid of a social scientist. Unfortunately, there's rarely budget for that type of groundwork – and there isn't always the time. But we need to prioritize it much more than we currently do, and we need to make sure that we're engaging the community throughout our research projects.

The long term

Previously, and in a western context, "post-trial access" referred to ensuring that clinical trial participants could continue to receive the experimental treatment in the window between the trial completion and medicine registration. In LMICs, the problem is more complex: how can the country retain access to the medicine? Many treatments, upon reaching the market, are priced beyond the reach of these countries. There are positive examples of "access strategies," but they're all chosen by the research sponsors themselves. There's currently no system in place to ensure early and continued access at an affordable price to those in the host countries who need it – but, in my opinion, there should be.

Overcoming operational obstacles

In the end, many problems – and their solutions – come back to project management. In small academic groups, when we want to develop the capacity for clinical research, we invest in scientific and clinical practice skills. We often fail to prioritize investment in project management and administration – but those are the skills that make your research more efficient, and even more ethical. Without project management, your budget may be missing essential elements (such as preliminary cultural studies or the resources to upgrade existing facilities or engage the community) – and you can't amend your external budget after the fact, so you need to make sure it's correct from the start. Once your study is underway, you still need administrative skills. If you want to send samples overseas for testing, you'll need fair and transparent material transfer agreements. If you want to share your data, you'll need contracts that protect your rights, and the rights of your research partners and study population. These are all complicated matters, and they're all too easily overlooked if you don't have administration and legal experts. Never underestimate the value of good management!

And speaking of management, we have to remember that – unfair though it may be – some of us wield more power than others in our collaborations, and it's up to us to fight for those with less. When researchers from highly developed countries work with LMICs, we must not "take charge." It's the local scientists and doctors who have spent their lives getting to know the patients, the diseases, and the available resources – so why aren't we making sure they are the driving force in decision-making? And not just scientists and doctors; if you've spent any time in the field, you'll know that the people on the ground – nurses, community health workers, interpreters, data entry clerks – are all equally important. We can't view them as so many cogs in a machine. We need to support and involve them, and offer access to training and networking, so that they can continue to do their jobs and sustain their fundraising and research capacity, long after we've left.

Raffaella Ravinetto is a senior researcher at the Antwerp Institute of Tropical Medicine (ITM)'s Public Health Department, chairperson of the Médecins Sans Frontières Ethics Review Board, and former head of the ITM's Clinical Trials Unit.

This piece is based on material previously published in international peer-reviewed journals.

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