

Institution: University of the West of Scotland
Unit of Assessment: 3
Title of case study: Improved Eye Care Solutions and Medicines for the Prevention and Cure of <i>Acanthamoeba</i> keratitis.
<p>1. Summary of the impact (indicative maximum 100 words)</p> <p>Dr Fiona Henriquez's research into the debilitating eye infection <i>Acanthamoeba</i> keratitis (AK) has significantly impacted on the handling of contact lenses and their cases to the benefit of the world's 125 million contact lens users. Her research findings have been ground-breaking for the research community and have featured widely in the media reaching audiences from scientists, industry professionals, the general public and individual contact lens users. The research has resulted in 2 patents and there are 3 non-disclosure agreements in place with companies relating to commercialisation projects. Related work on <i>Acanthamoeba</i> cysts has resulted in a fourth commercialisation project.</p>
<p>2. Underpinning research (indicative maximum 500 words)</p> <p>Dr Fiona Henriquez's research interests focus on infections by opportunistic eukaryotic pathogens and in particular, the <i>Acanthamoeba</i> species. These microorganisms are difficult to treat due to the inefficacy of current medicines and the resistance of <i>Acanthamoeba</i> to many compounds. Some compounds that have been shown to be effective are also highly toxic to human cells. This pioneering research, undertaken in partnership with the University of Strathclyde (active since her appointment at UWS in 2005), led to the initial steps taken to develop a novel high-throughput assay system to assess the efficiency of the inhibitory compounds (Ref 3.1). This assay has facilitated Dr Henriquez's current research.</p> <p>In 2008 Henriquez et al., published a study that clearly presented the challenges faced in the treatment of <i>Acanthamoeba</i> keratitis (AK). In this research, the resistance of <i>Acanthamoeba</i> to several anti-tubulin compounds, including potent anti-neoplastics (paclitaxel, vinblastine) is described, thus illustrating its resilience to the most potent inhibitors to eukaryotic cells. This is despite the fact that tubulin is one of the most conserved proteins between species and the high resistance phenomenon was found to be due to different amino acids in critical binding areas of the <i>Acanthamoeba</i> protein. Significantly, these amino acid differences may be exploited in future studies to create a specific compound that can target only <i>Acanthamoeba</i> tubulin, thus reducing the toxic effects in the human host (Ref 3.2). This early research was followed up with in 2009 when Henriquez et al., described the characterisation of alternative oxidase (AOX), which is present in <i>Acanthamoeba</i> but not in the human host. AOX is a mechanism through which <i>Acanthamoeba</i> can overcome inhibitory pressures from toxic compounds and therefore it is important to take this into consideration for future drug design (Ref 3.3).</p> <p>Dr Henriquez's work has also focused on exploiting metabolic differences between <i>Acanthamoeba</i> and the human host and she has directed three UWS PhD students in this area. This work has characterised the histidine biosynthesis and shikimate pathways present in <i>Acanthamoeba</i> but absent from the human host. Her work has demonstrated that <i>Acanthamoeba</i> growth can be inhibited by compounds that block either of the pathways and has already been protected by two published patents.</p> <p>In addition to the challenge of the development of an effective treatment, investigation into contact lens user compliance highlighted the issue that the contact lens end user must be adequately informed of the potential risks of <i>Acanthamoeba</i> contamination of contact lenses. For this reason, Dr Henriquez organised an event at the British Science Festival in 2012 to highlight the potential risks of microbial contamination in contact lenses to the public. She brought together a multidisciplinary team of four experts; an optician who delivered tutorials about the correct use of contact lenses; an engineer to explain why contact lenses and their cases are ideal surfaces for microbial growth; an immunologist with expertise in immune responses in the eye; and a microbiologist (Dr Henriquez), who focused on the challenges faced in treating <i>Acanthamoeba</i> infection (Ref 3.4).</p>

3. References to the research (indicative maximum of six references)

3.1 McBride J, Ingram PR, Henriquez FL, Roberts CW. Development of colorimetric microtiter plate assay for assessment of antimicrobials against *Acanthamoeba*. J Clin Microbiol. 2005 Feb;43(2):629-34.

This publication is in a highly ranked journal in the subject area (number 3 in citations and number 7 ranked by Eigenscore). It has revolutionized how Acanthamoeba viability is assessed. Before this publication Acanthamoeba assessment was slow and reliant of the fact that Acanthamoeba can feed on bacteria. This new method is suited for high through put assays without the need for a co culture of organisms. Since its publication in a subject focused journal it has been cited 37 times and it is now routinely used in laboratories worldwide, including the CDC.

3.2 Henriquez FL, Ingram PR, Muench SP, Rice DW, Roberts CW. Molecular basis for resistance of *Acanthamoeba* tubulins to all major classes of antitubulin compounds. Antimicrob Agents Chemother. 2008 Mar;52(3):1133-5.

This publication is in the number 1 journal in pharmacology and pharmacy for both citations and by Eigenfactor score ranking. It is the first study into genetic factors in Acanthamoeba that influence its resistance to toxic molecules that are used as herbicides, anti-neoplastics and anti-helminthics. Since its publication it has been cited 15 times in journals concerning medicinal chemistry and biotechnologies at an international level.

3.3 Henriquez FL, McBride J, Campbell SJ, Ramos T, Ingram PR, Roberts F, Tinney S, Roberts CW. *Acanthamoeba* alternative oxidase genes: identification characterisation and potential as antimicrobial targets. Int J Parasitol. 2009 Nov;39(13):1417-24. doi: 10.1016/j.ijpara.2009.04.011. Epub 2009 May 21. PubMed

This publication is in a highly respected journal for parasitology (ranked 6 out of 63, Eigenscore). It is specific to Acanthamoeba research and at the time of publication offered a unique insight into the makeup of the Acanthamoeba genome, including intron mapping and the existence of polymorphisms.

3.4 http://www.abdn.ac.uk/2012/documents/BSF_PublicProgramme_FINALFOREMAIL.pdf
Evidence of participation at BSF and this itself was an investigative research project to increase understanding of the public's knowledge of Acanthamoeba.

4. Details of the impact (indicative maximum 750 words)

There are 125 million contact lens wearers worldwide and potentially all are at risk of *Acanthamoeba* infection and therefore this research has a global impact.

Following publication of her research findings on the *Acanthamoeba* keratitis in 2008, Dr Henriquez actively engaged in dialogue with the commercial arm of the world famous Moorfields Eye Hospital NHS Foundation Trust, Moorfields Pharmaceuticals. The company specialises in the manufacture and development of sterile liquid products and Ophthalmic Specials products. Moorfields agreed to conduct a survey amongst Ophthalmologists, which confirmed the unanimous agreement that an effective pharmaceutical product was required to treat *Acanthamoeba* infection.

The impact of this research has been in 4 areas:

a) **Commercialisation and Industry Impacts:** Through publication of patents and participation in industry-focused conferences, Dr Henriquez has been involved in discussions with several eye care companies. To date, non-disclosure agreements (NDA) have been signed by 3 separate companies: Boots Technical Centre for Boots Opticians, which has almost 700 UK ophthalmic and dispensing optician stores and circa 5000 staff; Viopti, which is a small to medium sized enterprise (SME), registered as a company in May 2009; Sauflon Pharmaceuticals Ltd, a global manufacturer of contact lenses and solutions, offering the widest range of contact lenses and aftercare products anywhere in the world. Sauflon's high-quality contact lenses and aftercare

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products reach Opticians, Optometrists, Eye Doctors and Eye Care Professionals in over fifty countries.

- b) **Patents Awarded:** Dr Henriquez's research has resulted in Patent awards for both Antimicrobial and Antiprotozoal compounds (See Section 5.1). The patents protect the use of the inhibitory compounds glyphosate and 3-amino-triazole that block the shikimate pathway and histidine biosynthesis pathway in *Acanthamoeba*, respectively. Patents were awarded in Europe and in the patent covering the shikimate pathway has also been awarded in the USA.
- c) Sauflon, who have signed a NDA, have seen the patent and have already tested the shikimate pathway inhibitor, glyphosate, with their contact lens solution "All-In-One Light". This resulted in an increased efficiency of this contact lens solution to eliminate *Acanthamoeba* trophozoites from contact lenses. Investigations are now focusing on elimination of the highly resistant cyst form within 6 hours.
- d) **British Contact Lens Association new warning labels on contact lens cases:** In September 2012, Dr Henriquez organised the event 'Microbes in contact lens' at the British Science Festival in order to highlight the dangers of contact lens negligence and poor care and that finding effective medicines for *Acanthamoeba* keratitis is a considerable challenge as they are eukaryotic (as are humans) and thus many treatments are toxic to humans. This research into finding new protective measures and treatments, through public engagement and media discussion and consequent correspondence (Section 5), encouraged an artist/patient to design dedicated warning labels for contact lens solution boxes and cases to inform users to avoid water exposure of their contact lenses (Section 5). These 'no water' sticker labels have been endorsed by the British Contact Lens Association (Section 5.1). The BCLA are distributing these labels to their members and the stickers are also available to purchase. Dr Henriquez has received letters and emails from members of the public endorsing the research, or seeking information and advice on contact lens handling and care (Section 5.3). This is evidence of changing behaviour and perception in the end user of their own use of contact lenses and thus significant impact on improving public health.
- e) In 2012 Dr Henriquez received a Scottish Funding Council Innovation Voucher Scheme award to work with the microbiological biocide testing company, Blutest. Currently, there is no international standard (ISO) specifically detailing procedure to assess the efficacy of biocides/contact lenses solutions against *Acanthamoeba*. Therefore, the aim of this project was to develop a rapid test to assess biocides on highly resistant protozoan cysts. Cysts are notoriously difficult to test since they have extremely low metabolic activity and do not replicate. The test, which is based on monitoring excystation by a colourimetric assay, has been validated through testing several biocides and can now be used to evaluate the efficacy of biocides, which is already of commercial impact for Blutest (See Section 5.1).
2. **Media Article Impacts:** Relevant ophthalmic professional bodies have sought interviews with Dr Henriquez, with resulting articles in specialist industrial journals in Ophthalmology, such as 'Optician' magazine, a key reference for Optician practitioners. There has also been significant public dissemination through national and international media portals, including The Daily Telegraph, The Daily Mail, MSN and yahoo news. (See Section 5.2). The public have responded to these articles through online discussions, some highlighting their own experiences with the disease and some calling for more awareness of this pathogen.

5. Sources to corroborate the impact (indicative maximum of 10 references)

5.1. COMMERCIALISATION/INDUSTRY IMPACTS

- PATENT: Roberts, C.W. Ingram, P.R. HENRIQUEZ, F.L. & Roberts, F. (2006). Antimicrobial Compounds PCT/GB2006/000875.

This patent describes the utilization of the shikimate pathway in *Acanthamoeba* as a target to

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stop *Acanthamoeba* growth. It protects the use of glyphosate in contact lens solutions and as a therapeutic agent.

- PATENT: HENRIQUEZ, F.L & Roberts, C.W. (2008). Antiprotozoal compounds. UK patent application PCT/GB/2009/051197.

This patent protects the use of the histidine biosynthesis pathway in *Acanthamoeba* as a target to inhibit *Acanthamoeba*. It protects the use of 3-amino-triazole in contact lens solutions.

- BCLA Adoption of new “no water” labeling and letter from the Artist, who was inspired to design these labels from the ‘Microbes in contact lenses’ event at the British Science Festival 2012.
 - <http://www.bcla.org.uk/en/news/index.cfm/No%20water%20stickers>
 - http://www.opticianonline.net/opticianspace/blogs/the_contact_lens_blog/archive/2013/07/04/sticking-with-no-water.aspx
- Boots Technical Centre
Non-disclosure agreement and letter of interest 2009
- Viopti
Non-disclosure agreement, SeeKIT innovation voucher and letter of support 2009
- Sauflon Pharmaceuticals Ltd
Non-disclosure agreement.
- Blutest -SFC IVS voucher scheme report on work leading to successful rapid assay development.
Letter of support

5.2. MEDIA ARTICLE IMPACTS

All media articles are also available online and this has stimulated public discussion about *Acanthamoeba* and its devastating effects.

- <http://www.dailymail.co.uk/health/article-2199343/Contact-lens-wearers-mercy-bug-tap-water-gnaws-eyeballs-causing-blindness.html>
- <http://au.lifestyle.yahoo.com/health/diet-nutrition/article/-/14781178/people-with-contact-lenses-at-risk-of-eye-eating-parasite/>
- <http://news.uk.msn.com/health/amoeba-alert-to-contact-lens-users>
- <http://www.belfasttelegraph.co.uk/news/health/contact-lens-wearers-at-risk-of-amoeba-that-can-eat-eyes-28789850.html>
- <http://www.healthcareglobal.com/administration/save-your-contact-lenses-from-a-rare-parasite>

5.3. GENERAL PUBLIC IMPACTS

Example Quote from member of public dated 8th September 2012 ‘My wife was the second reported case in the United States approx 26 years ago. We assume she contacted it from the water in a Hot Tub. She also wore contacts at the time. She did have to have a Cornea Transplant – It was done out of UCLA. I found this article very similar to the symptoms she (my wife) had. She is doing well now, has lost some peripheral vision and will most likely go blind in her one eye. Good luck and continue success with your studies. Please feel free to contact me if we can help in anyway’