<u>**Laidlaw Programme Reflective Report**</u>

<u>**Scholar Report**</u>

Name:	Ryan Oatley
Faculty:	School of Chemical and Process Engineering
Email address:	Pm22ro@leeds.ac.uk
Title of Scholarship Project:	Nanoparticle-based therapeutics to combat multidrug resistance and biofilms in chronic wound infections

Research conducted:

This summer, I have undertaken laboratory-based research work in the Bragg building, working under Dr Zhan Ong's supervision and with postgraduate students to conduct my research project. This project involved conducting nanoparticle synthesis and utilising various characterisation techniques, to allow the incorporation of antibiotics into the organic shell of gold nanoparticles (AuNPs) to then be formulated into a hydrogel. Light activation can then be used to release the antibiotics and thermally eradicate bacteria on the infection site, preventing antibiotic resistance and the formation of biofilms. This was comprised of both active and passive laboratory work, i.e. setting up an experiment to be left for several hours throughout the day. Therefore, to make the most of my time, I performed independent research on this project to aid my understanding of the practical work. Primarily, this consisted of a literature search and review to initiate the research project.

The literature search used the key terms (Au) Nanoparticle, Chronic wound infections, Hydrogel, Biofilms, Localised Surface Plasmon Resonance (LSPR), Antibiotic resistance, Polycatechin, Nanoparticle Synthesis, Staphylococcus epidermidis, and Drug Delivery, using academic search engines such as google scholar and PubMed. Approximately 20-30 papers were collected and analysed for background reading, and a systematic literature review was produced, providing context for various topics involved in the research, and identifying limitations in current literature research. This showcased the areas best to specialise this research into, to avoid repeating previous studies, e.g. assessing biofilms with multiple pathogenic species rather than a single species of bacteria, as commonly seen in the literature

Following the literature review, I received a laboratory induction to ensure good laboratory practice (GLP) and familiarised myself with the Bragg Building and its facilities. Working under the supervision of PhD student Xinyu Mao, we initiated the synthesis of AuNPs to be characterised further into the project. We followed the process conducted by a previous student's master's project, where the AuNPs produced were comparable to those in the project. Zetasizer and UV-Vis machines were used to characterise these NPs, determining size, and wavelength for peak absorbance. Initially, I shadowed Xinyu as she conducted the nanoparticle synthesis, making notes in my laboratory notebook. Also, throughout all experiments, I adhered to the strict health and safety protocols regarding wearing PPE including lab coats, goggles, and gloves, as well as safe disposal of chemicals and equipment.

Following the shadowing, I conducted my own synthesis under Xinyu's supervision, allowing me the opportunity to use equipment such as the balance, pipettes, ultrasound and vortex emulsifiers, and centrifuge machine. Then, I repeated this process independently, gaining key laboratory skills. This time, I created two separate samples of varying Au concentration, with larger concentrations used. This is to get closer to the goal initially set of 800nm absorbance, which is necessary for photothermal treatment, so that the thermal eradication of bacteria to take place. Next, a silver loading process was undertaken to utilise their synergistic effects and increase the overall therapeutic potential, and this also enabled the comparison to non-loaded samples. Finally, transmission electron microscopy (TEM) was used to image these nanoparticles.

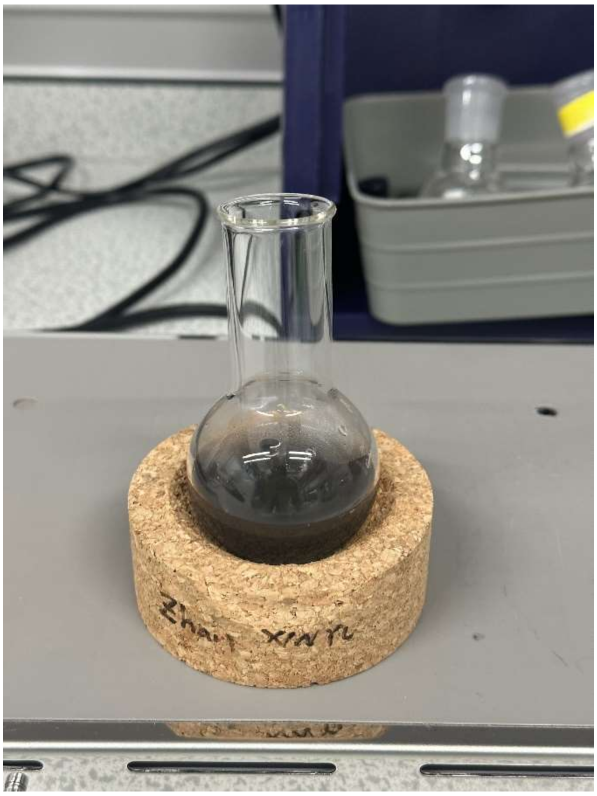

Figure 1 - AuNP/CH synthesised solution, pre centrifugal purification

Importance/impact of the research

Regarding the wider context of why this research is being conducted, wound infections are prevalent in a growing, geriatric population. In a skin wound, once the fatty subcutaneous layer of skin is compromised, protein and lipid denaturation and outer epidermal interference provides a rich ecosystem for bacterial growth. Whilst wounds tend to self-heal, via the phases of haemostasis, inflammation, proliferation, and remodelling, the retardation of healing and prolonged inflammation may cause an infection to persist. Moreover, key risk factors including diabetes, where cases are predicted to rise from 463 million (2019) to 700 million (2045), will increase demand for treatment and can even cause cases to become life-threatening. This has led to an enormous rise in the global advanced wound dressing market, currently valued at $6.85 billion with a CAGR of 4.3%. [1]

Furthermore, the use of antibiotics to combat bacterial infections first began in the 20th century, when penicillin was first produced in the 1940s. Current antibiotics range from topical ointments e.g. Neosporin, intravenously injected solutions, and oral medication. They comprise of chemotherapeutic agents that kill microbes and restrict growth, by inhibiting cell wall synthesis, protein synthesis, nucleic acid synthesis, metabolic pathways, and membrane integrity. [2] Whilst antibiotics are effective in causing metabolic imbalances in bacteria, resistance to these antibiotics is a major issue, as 70% of wound colonising bacteria have demonstrated resistance to at least 1 frequently prescribed agent. [1] The rise in multidrug resistant (MDR) bacteria has increased medical treatment costs, as $20 billion is spent annually worldwide. As 300 million people are affected by wound infections, a further $25 billion is spent in the US alone, causing major financial stress to healthcare systems. [3]

Common resistive bacteria include staphylococcus aureus, methicillin-resistant staphylococcus aureus (MRSA), streptococcus pyogenes, and pseudomonas aeruginosa [4], with over 40% of staphylococcus aureus being methicillin and vancomycin - the latest generation of antibiotics most effective for staphylococcus aureus - resistant. [2] Antibiotic resistance occurs by restricting entry of antibiotic molecules, altering antibiotic targets via mutation or posttranslational modification and protection, or resistance due to direct hydrolysis and chemical group transfer upon the antibiotics, which is the primary resistance mechanism. Whilst almost all bacteria are prone to resistance, gram negative bacteria are less permeable to antibiotics due to their selectively permeable membrane. This research will focus on the second most commonly reported bacterial species, the gram positive staphylococcus aureus (20.6% of reported cases), where MRSA cases specifically have declined from 19% (2015) to 15.5% (2019). [5] Although, combined resistance to another antimicrobial group is common.

Antibiotic resistance of bacterial biofilms is responsible for 60% of chronic infections [6], and 70% of western world infections are biofilm associated. Biofilms are a group of microbial cells adhered to a surface and embedded in a self-produced extracellular matrix made of extracellular polymeric substances (EPS), including proteins, exopolysaccharides, dead bacteria and bacterial DNA, and enzymes. This acts as a protective barrier against antibiotic penetration and natural immune cells. As a result of this, biofilm bacteria are 10-1000x less sensitive to treatments with antimicrobial agents compared to planktonic cells. The EPS limiting diffusion and chemical reactions with agents places an urgent need for antimicrobial treatment that efficiently disrupts biofilms and is non-toxic to host tissue in infection clearance and wound resolution, [7] as the chronicity of non-healing wounds is associated with increased incidences of biofilm formation.

Activities to disseminate my research

To disseminate my research and explore wider ethical issues associated with biotechnology, I partook in a Health Equity Hackathon, held by pharmaceutical companies MSD and Eli Lily in London. This event focused on developing innovative solutions for various global issues, with my specific topic encompassing different methods to improve diversity in clinical trials for breast cancer therapies. Whilst groundbreaking research work in the biotechnology space is crucial for further advancements in medicine, clinical trials are just as important, and significantly low engagement from ethnic minorities has often led to disparities in treatment efficacy across different ethnic backgrounds. With no clinical trials for antibiotic AuNPs to date, this is a very relevant subject area for my current research. I worked with a team of fellow STEM students and graduates to present an AI-based solution to combat this issue, enhancing my communication skills as I worked with a team of new people, and my public speaking skills as I presented to management level staff from both companies.

Figure 2 - My MSD/Lily Hackathon team

In addition, this event was an incredible opportunity to network with like-minded students from a range of fields, and employees from MSD and Lily. The relevance of my research enabled me to discuss this with others present and promote the Laidlaw program, and my attendance has provided me with a 'fast track' route when applying for placement year roles, enhancing my future career prospects. Therefore, not only did I develop key leadership attributes such as teamwork, communication, and public speaking skills, but could apply these skills in a professional manner to potentially aid my future career path.

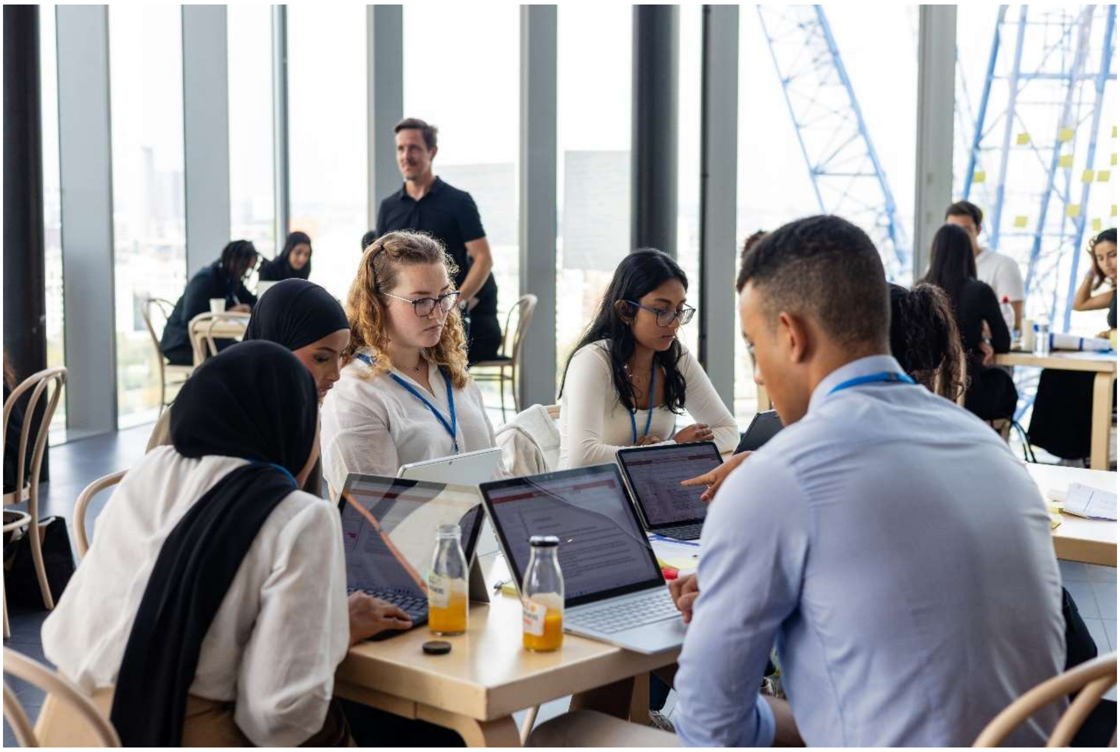

Figure 3 - My MSD/Lily Hackathon team forming our presentation

Future career plans

This project has further developed my interest in working in either a research and development or chemical engineering role in the pharmaceutical/biotechnology industries, as the knowledge I have gained in complex biological concepts, nanotechnology, and laboratory skills provides an amazing foundation for further work in related fields. The independent research skills used in the Laidlaw project will be further utilised in my degree coursework as I enter my second year, but even more so when I undertake my master's degree in my 4th year.

Currently, I wish to gain a placement year role in either the pharmaceutical or energy sectors, and then progress to work in a chemical/process engineering role upon graduation. It must however be noted that the versatility of a chemical engineering degree means this is very much subject to change, as the problem solving skills gained in a highly quantitative engineering degree can be utilised towards a range of fields, including finance and technology – both fields which I am also very much interested in. Experience outside of my degree such as the Laidlaw research project enables me to not only broaden my academic knowledge but gain new skills which can support a transition into different industries for my future career. Crucially, the Laboratory skills and techniques equipped from this project are applicable to a plethora of roles in the life sciences, biotechnology and pharmaceutical industries, further widening the opportunity for work post-graduation.

How conducting research has impacted me

The research period arrived during a busy summer, as I initially worked at the Harwell Science and Innovation Campus for an internship with a nuclear fusion company, Fusion Energy Insights. During June, this internship coincided with the two Laidlaw residentials, at the Village Hotel and the Yorkshire Dales respectively. Travelling from Harwell to London, and then to Leeds and back each week required discipline and determination, as developed my time management skills when managing early mornings and late nights to travel around a busy work schedule. In addition, and perhaps an underestimated leadership quality, preparation was key in this instance. By adopting a ’first in, last out’ mentality, this enabled me to complete required work in advance, avoiding unnecessary, last-minute stress. This was also demonstrated during a 5-week research program at KAIST, South Korea, where I also worked for a food poverty social enterprise outside of my working hours. Furthermore, the KAIST research was also in the field of nanotechnology and provided a strong foundation in relevant topics such as Localised Surface Plasmon Resonance (LSPR), and NP characterisation methods, such as various microscopy techniques.

As I returned from South Korea, I resumed my FEI internship and remotely worked full-time alongside my research. This forced me to maximise my time and further develop time management skills by setting deadlines for tasks and using SMART goals to organise my schedule. Whilst there may be a concern regarding ‘burnout’ before the semester begins in October, I believe that my busy summer has preset a strong and productive work rate which will be required to excel in my studies. Moreover, additional benefits such as experience for the CV, and the relief of financial stress for the upcoming year, only further solidify how important this past summer was for both my personal and professional development as a leader.

Supervisor

Please comment on your scholar's research period, what you consider to be your scholars' strengths and which leadership attributes you feel your scholar has demonstrated and is particularly skilled in. You could also identify areas which your scholar can develop further.

Ryan has shown good initiative throughout the placement. He demonstrated his ability to work independently and conduct literature review on the research topic during the first 1-2 weeks of the internship. Having just returned from a research internship from South Korea, he has already acquired the skills and knowledge to do so, which is impressive for a first year undergraduate student. He has also conducted himself professionally in his interactions with the PhD students and I. He responds promptly to emails and communicates clearly verbally and in writing. He has shown good project and time management skills by being able to plan and execute experiments around his other training activities such as the hackathon event. He also took the initiative to update me on his progress throughout the placement, even during weeks while I was away from the office. His positive attitude towards his work, resourcefulness, ownership of his own development and collegiality will put in good stead as he acquires a stronger foundation in the Engineering and Physical Sciences during the course of his remaining studies at Leeds.

initiative

Signature of Scholar ____Ryan Oatley__ Date: 25/09/2023

Signature of Project Leader ___Zhan Ong____________Date: 28/09/2023

References

[1] Jiang, L., & Loo, S. C. J. (2020). Intelligent nanoparticle-based dressings for bacterial wound infections. *ACS Applied Bio Materials*, *4*(5), 3849-3862.
[2] Ranghar, S., Sirohi, P., Verma, P., & Agarwal, V. (2014). Nanoparticle-based drug delivery systems: promising approaches against infections. *Brazilian Archives of Biology and Technology*, *57*, 209-222.
[3] Makabenta, J. M. V., Nabawy, A., Li, C. H., Schmidt-Malan, S., Patel, R., & Rotello, V. M. (2021). Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. *Nature Reviews Microbiology*, *19*(1), 23-36.

[4] Xu, C., Akakuru, O. U., Ma, X., Zheng, J., Zheng, J., & Wu, A. (2020). Nanoparticle-based wound dressing: recent progress in the detection and therapy of bacterial infections. *Bioconjugate Chemistry*, *31*(7), 1708-1723.
[5] Okkeh, M., Bloise, N., Restivo, E., De Vita, L., Pallavicini, P., & Visai, L. (2021). Gold nanoparticles: can they be the next magic bullet for multidrug-resistant bacteria?. *Nanomaterials*, *11*(2), 312.
[6] Chapter 46 - Nanoparticle-based treatment of bacterial biofilms
[7] Kim, M. H. (2016). Nanoparticle-based therapies for wound biofilm infection: opportunities and challenges. *IEEE transactions on nanobioscience*, *15*(3), 294-304.