Biological evaluation of Biomimetic Polyurethane-grafted Poly(ionic liquid) Bromide patchy colloidal particles to combat antibiotic-resistant Staphylococci bacteria (ESR 15)

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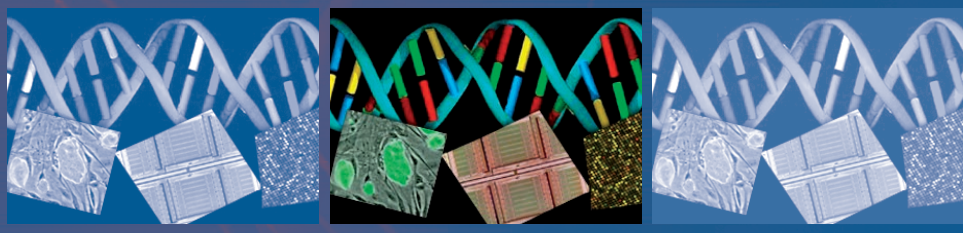

Drug-Free Antibacterial Technology for Medical Applications
First Cambridge International Conference

December 14th, 2018
The Hauser Forum, Cambridge, UK

Abstract Book

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Ipsita Roy
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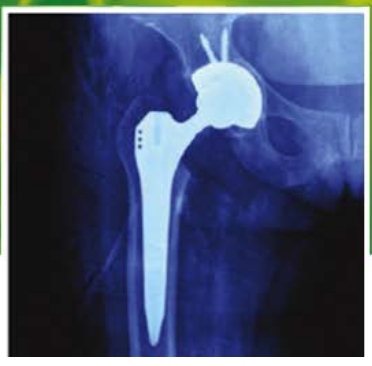

HyMedPoly received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 643050.

HyMedPoly

The HyMedPoly Project: New Concept of Drug-free Antibacterial Science Technology

Due to increasing prevalence of drug-resistant bacteria and as the number of effective antibiotics continues to shrink, finding new methods of combating infection has become one of the largest problems in the medical world. There is also growing consensus that biomedical polymers of both natural and synthetic origin can and should play a larger role in the development of new therapeutic strategies to treat disease. This special issue reports on a new development in the war against bacterial infection: "Drug-Free Antibacterial Hybrid Biopolymers for Medical Applications". This major project has been carried out under the EC scheme of Marie Skłodowska-Curie Actions Innovative Training Networks and results from the work of 15 PhD students and over 30 professionals from different universities and industries, all of whom have worked incredibly hard over the last 4 years to develop this innovative approach.

The aim of the project was to design and develop a series of new materials incorporating combinations of synthetic and natural polymers, bioglass and bioceramics which have been equipped with antibacterial functionality or with inhibitors that can permanently deactivate bacterial proteases.

Biomedical polymers have already become widely used in combination with drugs and active principles in medical applications, such as wound care, implants and bio film prevention. Our challenge was to develop new medical polymers that have an intrinsic antibacterial functionality to achieve clinical effectiveness in the field. This project took on that challenge and has successfully developed new materials that could provide practical medical solutions without requiring them to be paired with drug treatments.

When looking for ways to design new materials, our team devised three main strategies. These have guided the progression of our project and resulted in successful development of a series of novel therapeutic hybrid polymers to combat bacterial-related infection:

Developing new hybrid polymers (both synthetic and natural) provided with antibacterial functionality;

Designing new inorganic molecular structures with antibacterial properties;

Building new materials which incorporate natural inhibitors that can permanently deactivate bacteriological proteases

Each of these concepts can be used individually or in tandem when designing materials.

I would like to take this opportunity, as coordinator of the project "Drug-Free Antibacterial Hybrid Biopolymers for Medical Applications", to firstly thank our 15 PhD students who have all worked hard to make contribution to this project: Jeddah Marie Vasquez, Subha Purkayastha, Lukas Gritsch, Binh Thi Thanh Phan, Elena Marcello, Isabel Orlando, Seray Kaya, Mahammad Maqbool, Agata Lapa, Alexandra Paxinou, Sheila Piarali, Faezeh Shalchy, Loris Domenicale, Ayesha Idrees and Patricia Varela.

I would also like to express my sincere thanks to both our academic and industry supervisors, without whom it would have been impossible for this project to succeed: Professors Ipsita Roy (Scientific Coordinator), Gianluca Ciardelli, Valeria Chiono, Aldo Boccaccini, Atul Bhaskar, Wenxin Wang, Jochen Salber, Doctors Mark Cresswell, Chris Lovell, Philip Jackson, Ian Campbell, Iban Quintana, Maria Joao Barros, Hongyun Tai, Ellen Tallas.

Special thanks also go to the project manager, Dr. Stuart Maclachlan of Lucideon, who has made a great effort to keep the project running smoothly and productively.

Finally, I would like to thank the Marie Skłodowska-Curie Actions Innovative Training Networks H2020-MSCA-ITN-2014 for their financial support toward this "Drug-Free Antibacterial Hybrid Biopolymers for Medical Applications" project.

Prof. Dr. Xiang Zhang

Principal Consultant at Lucideon
Royal Society Industry Fellow at the University of Cambridge

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HyMedPoly

The Art of Commercialising Research

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Prof Lowe outlined his experience of working with companies to commercialise innovative bio-based technologies and the important aspects for successful entrepreneurship including financing methods, their associated risk and the personal traits of the entrepreneur. The key steps in business planning and the process and cost of bring products to various market segments from computer apps to new drugs and airliners were then described.

Clinical Need and Issues Faced by Drug-Free Antibacterial Technology

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An increasing antimicrobial resistance (AMR) leads to a dangerous threat to the European public health requiring measures across all economic and social areas. The success of major surgery and interventional therapies and the necessary application of modern biomaterials-based implants and medical devices depends on the availability of highly potent antibiotics and/or alternatives. Different reasons led to this AMR crisis.[1] The reasons are:

1. Global misuse of antibiotics by laymen and professionals similarly;
2. Rudimental or partially completely missing hygiene standards in healthcare;
3. "Professionals" (e.g. MDs, nurse staff) are the main cause, directly and indirectly;
4. Senseless cost reduction or ignoring necessary investigations by the hospital operating company;
5. Strong increase of the application of implants, endoprostheses and medical devices and thus the potential danger of foreign material-associated infections;
6. Criminal error not to continue research and development of new antibiotics caused by governments, health insurances and pharmaceutical industry;
7. Lacking knowledge about mechanisms of antimicrobial resistance in the context of Foreign Body Reaction (FBR)-associated colonisation, biofilm formation and infection;
8. Missing or retarded clinical implementation of alternative, complementary, drug-free antimicrobial strategies.

Antimicrobial-modified biomaterials will play an indispensable role in modern medicine in form of implants, wound dressings and tissue engineering approaches to prevent particularly bacterial adhesion and biofilm formation. The prevention of microbial attachment to implant surfaces and the inhibition of their proliferation and organisation in biofilms on inner and outer surfaces of medical devices lead to the critical question regarding their biocompatibility. The awareness of requirements for testing biocompatibility correctly, comprehensively and carefully can prevent the industry from making common mistakes that could slow a product time to market, the academics from giving clinicians and patients wrong hope of contemporary help, the hospital operators and clinicians from buying and applying products only tested by inadequate standards and finally the patients from harming by worse medical products and treatments.[2] We suggest hereby a more comprehensive and advanced approach on how modern bioactive, antimicrobial and biofilm-preventing biomaterials should be bio-evaluated: starting with rudimental haemo- and cytocompatibility *in vitro* tests, a panel of new biomaterials and biomaterial surface modifications should be tested according to the latest DIN EN ISO standards 10993-4/2017-12 and -5/2009-10 with the aim to provide a shortlist of the panel and finally to save time and money. Depending on the final application, advanced in vitro bio-evaluation should follow by studying the interaction between shortlisted biomaterial candidates and primary cells. We prefer to follow then two separate routes, one route using primary animal cells and another applying primary

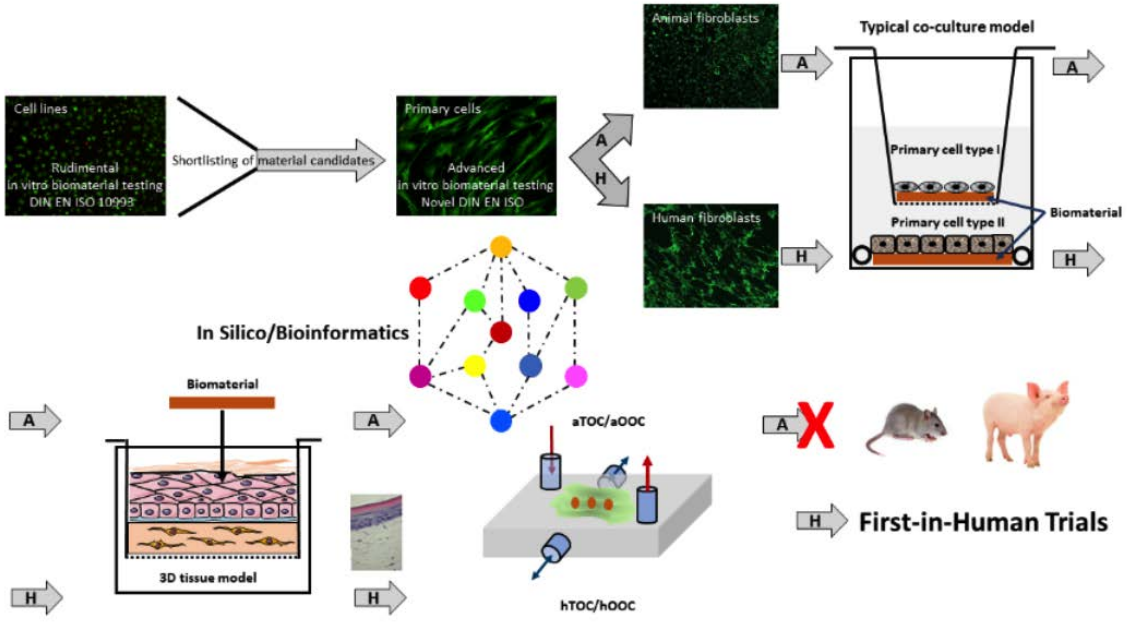

Figure 1. A prospective route of a more reliable and valid pre-clinical bio-evaluation of novel biomaterials and medical device prototypes – Shortening the timescale from bench-to-bedside, reducing implant failures, saving money and senseless sacrificed animal lives.

human cells with respect to the target tissue of the final implant or medical device. Finally, the first route serves as an *in vitro* pre-test platform applying haemo-, cyto- and immunocompatibility analyses with the aim to reduce the number of *in vivo* small and particularly large animal trials according to the intentions of the European Union Reference Laboratory for alternatives to animal testing and European Centre for the Validation of Alternative Methods (EURL ECVAM). The second route serves as a parallel complementary approach to compensate deceptive results based on interspecies differences in particular regarding immunology. Furthermore, as many other academic and industrial research groups we started working on co-culture, multi-culture models and finally 3D human tissue equivalents.[3,4] In this context, novel approaches like human cells-derived tissues-on-a-Chip (hTOCs) and Organs-on-a-Chip (hOOCs) as miniaturized *in vitro* human tissues and organs[5] serve as the next promising step to investigate *in vitro* biocompatibility of novel multi-functionalized biomaterials (*e.g.* drug-free antimicrobial biomaterials), drug delivery systems, implants and medical devices. Once these techniques are combined with *in silico* analysis tools (bioinformatics) we would be able to generate more reliable and valid pre-clinical data without sacrificing even one animal. Figure 1 shows schematically the current and prospective promising route of novel pre-clinical *in vitro* biocompatibility evaluation.

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Biomimetic Strategies in the Design of Antibacterial Synthetic Polymers

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Introduction

Learning from Nature is an increasingly winning strategy in the design of functional materials and device in a wide range of final applications. In particular, this approach finds its obvious exploitation in the biomedical sector. In this context, a challenging goal is to develop novel medical polymers that have an intrinsic antibacterial functionality to tackle the increasing occurrence of microbial infections. The biomimetic strategy finds here an application in designing building blocks that mimes part or whole natural antibacterial materials. To this aim, the development of different polymers, which mimic respectively honey and antimicrobial peptides have been explored in the HyMedPoly project.

Research Highlights

a. To address the inability of therapeutics to reach the site of invasion of pathogenic bacteria in chronic wounds leading to persistent infection, a biomimetic polyurethane-grafted poly(ionic liquid) based patchy anisotropic colloidal particles for topical and intradermal wound healing applications mimicking the behaviour of Antimicrobial peptides (AMPs) was designed. AMPs are essential components of immune system forming the first line of defence against pathogenic bacteria. A hydrophobic liquid monomer was grafted from the polyurethane backbone by redox initiated aqueous heterophase polymerisation. Subsequently, the hydrophobic anion was exchanged with a hydrophilic one. Chemical structure was elucidated by NMR analysis. Cryo-TEM images confirmed the formation of patchy colloidal particles consisting of the self-organised mesophases. The particles showed a strong bactericidal effect.

b. A honey-like hydrogel was prepared via thiol-ene click chemistry of hyperbranched polyethylene glycol diacrylate and thiolated hyaluronic acid. The hydrogel produced antibacterial reactive oxygen species (ROS) in the form of hydrogen peroxide (H^2O^2), through the two components found in honey: glucose oxidase (GO) enzyme within HB PEGDA and glucose (G) in HA-SH. The hydrogel was able to produce 9.11 mmol H^2O^2 ROS after 24 hours with 250 U/L GO and 25 g/L G. This concentration demonstrated zone of inhibition as a measure of antibacterial activity against several bacterial strands.

Applications, Prototypes, Development

The sheer inability of therapeutics to reach the location of invasion of pathogenic bacteria in deep tissue layers or even in interstices of cells in tissue in case of chronic wounds, leads to persistent infection. To tackle this problem, the research approaches described are currently developed towards viable routes of administration:

a. The polyurethane suspension can be delivered topically or through an intradermal application.

b. An electrospun polyurethane patch was prepared and surface modified with polydopamine for the immobilisation of GO enzyme, to be used in combination with the above-described hydrogel.

With the collaboration of the industrial partners of the project, the ESRs are now seeking the best route for product development and technology qualification.

Antibacterial Natural Polymers

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Natural Polymers have the potential to be used in a variety of medical applications due to their excellent biocompatibility, varied mechanical properties and sustainable resourcing. These include polymers such as alginate, gelatin, chitosan, collagen, gellan gum, silk and chitin. In addition, there is a distinct class of natural polymers that are produced by controlled bacterial fermentation. These include amongst others polyhydroxyalkanoates (PHAs),[1] bacterial cellulose (BC),[2] γ-polyglutamate (γ-PGA) and alginate. The added advantage of this class of natural polymers include the highly controlled production conditions which lead to

repeatable properties, a requirement for most applications and more so for medical applications.

Research Highlights

PHAs are polymers of 3,4,5 and 6-hydroxyalkanoic acids produced by bacteria, mainly under nutrient limiting conditions. These are divided in to two main types, short chain length PHAs, with monomer chain length, C_4-C_5, which are normally hard and brittle in nature; and medium chain length PHAs, with monomer chain length, C_6-C_{16}, which are normally soft and elastomeric in nature. In this work we have modified PHAs with various natural antibacterial agents including antimicrobial peptides and active factors from garlic, among others. In addition, a naturally antibacterial class of PHAs, thio-PHAs have also been produced.[3] All of these antibacterial polymers have been characterised with respect to their antibacterial activity against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 following ISO22916. The biocompatibility of these materials have been assessed using C2C12, L929, and NG108, a myoblast, fibroblast and neural cell line respectively. These antibacterial polymers have been used for the development of tissue engineering scaffolds and medical devices.

BC is produced by several bacteria including *Gluconacetobacter xylinus* and has an inherent hydrogel-like structure. This particular feature enables it to incorporate more than 90% of its weight by water, thus providing an optimal level of moisture and turning the material into a natural wound dressing. Moreover, BC is highly pure as it does not contain lignin and hemicellulose, ensuring a great degree of biocompatibility without needing harsh purification treatments. In this work the surface of cellulose was functionalized by wet chemistry to introduce active groups. Antibacterial studies showed a decrease in the bacterial growth after 24 hours of contact with the samples. The cytotoxicity evaluation using HaCaT cells (keratinocytes) confirmed the cytocompatibility of both modified and unmodified BC.

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The Development of Antibacterial Inorganic Materials and their Potential for New Medical Technologies

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Introduction

The antibacterial capability of certain natural minerals and the elements within them is well known and this knowledge has previously been exploited to good effect. The incorporation of these potent antibacterial agents into inorganic-based therapeutic materials and medical devices presents promising new opportunities to combat the threat of widespread antimicrobial resistance (AMR) to conventional antibiotic treatments.

Research Highlights

Three distinct approaches have been investigated for the generation of inorganic materials with antibacterial properties: i) the preparation of ordered and non-ordered mesoporous silicate bioactive glasses doped with known therapeutically useful and antibacterial ions; ii) the preparation of multi-substituted hydroxyapatites doped with ions specifically for bioactivity and antibacterial efficacy; iii) the preparation of phosphate-based glasses and the analogous phosphate glass fibres doped with antibacterial ions.

By controlling the fundamental composition and structural properties, and by incorporating antibacterial ions in differing amounts into the three types of material, the relative physicochemical properties and antibacterial efficacy of the materials can be modulated. The most promising materials were characterised for their antibacterial efficacy using different *in vitro* methods against various Gram negative and Gram positive model bacterial strains. In addition, the cytotocompatibility of these materials were also assessed against different model cell lines.

Different approaches towards the production of antibacterial composite materials have been developed.

i) Incorporation of mesoporous bioactive silicate glass powders into polysaccharide (natural polymer) and polyurethane (synthetic polymer) thin films has been carried out for the production of novel antibacterial and wound-healing dressings.
ii) Electrophoretic deposition of suspensions of multi-substituted hydroxyapatite and chitosan onto metal coupons has been studied as a general method for the preparation of antibacterial and bioactive medical device coatings.
iii) The unique geometry of phosphate glass fibres supports their use as fillers for composite materials. A highly absorbent cellulose-phosphate glass fibre mesh material has been developed, again for the purpose of creating an antibacterial, wound-healing composite dressing.

All prototype product materials have been additionally tested for their antibacterial efficacy and cytocompatibility.

All three material types have demonstrated excellent antibacterial behaviour and cytocompatibility; work will now focus on further development and optimisation of suitable prototype product demonstrators.

Develop functionalized biopolymers products for cosmetic, pharmaceutical, biological and medical applications

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Blafar Ltd. has the expertise, knowledge and technologies in polymer design, synthesis and modification which can meet various needs for our global customers, particularly for cosmetics, personal care, pharmaceutical and biomedical/biological uses. Our vision and goal are to achieve the leadership position in functionalized polymers market by developing and manufacturing polymer products as key functional ingredients for the end use products as well as developing formulations of the end use products. Currently, functionalized polymers provided by Blafar Ltd includes PEG based synthetic biopolymers, and natural biopolymers and their functionalized derivatives such as (meth)acrylate hyaluronic acid (HA-A), thiolated hyaluronic acid (HA-SH), collagen and chondroitin. Moreover, Blafar Ltd has been actively collaborating with academic institutions to develop new functionalized biopolymers, such as the three PhD projects in collaboration with University of

Westminster and with University of Polito in HyMedPoly consortium on developing different types of drug free antibacterial materials, which include functionalized Polyurethane, functionalized cellulose and functionalized PHA using various modern chemistry approaches. These are all biocompatible polymers with designed functionalities to allow them to have intrinsic antibacterial properties.

Novel Antimicrobial Technology for Medical Devices, from BALI, IBIZA and PRINT-AID

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Introduction

Implant-associated infections are one of the major causes of implant failure. The pathogenesis of these infections involves two major bacterial strategies to protect themselves from antimicrobials and from the host defence. The implant provides the bacteria with a surface for adherence and biofilm formation. Within biofilms the bacteria are difficult to reach for antibiotics and host immune cells. Moreover, because of their low metabolic activity in the biofilm, bacteria are up to > 100-fold less susceptible to antibiotics. The second niche for bacteria is the peri-implant tissue. The combination of the implant as a foreign body material and the bacteria deranges functional antimicrobial immune responses and may down-regulate macrophage intracellular bactericidal mechanisms, allowing bacteria to survive in tissue and even within macrophages.

Research Highlights

In order to prevent and treat implant associated infections and avoid antibiotic resistance development, we developed novel antimicrobial strategies. In the BALI (Biofilm Alliance) consortium project we developed novel anti-biofilm agents in the form of highly potent novel Synthetic Antimicrobial and Antibiofilm Peptides (SAAPs). These SAAPs have broad spectrum, very rapid microbicidal activity and anti-biofilm activity, no detectable resistance development, activity in human plasma, and they prevent implant –associated infection in mouse and rabbit models, as well as skin biofilm infection by multi drug resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii.*[1,2]

In the IBIZA (Imaging of Biomaterial-associated Infection using Zebrafish Analysis) consortium project we developed a novel method to eradicate intracellular bacteria. Often used antibiotics such as gentamicin do not kill intracellular bacteria, since the bacteria are residing within phago(lyso)somes, whereas the antibiotics are entrapped in other endosomes. In order to "liberate" the antibiotics as well as the phagocytosed bacteria into the cytosol in order to allow the antibiotics to reach the intracellular bacteria, we developed a photosensitiser-based approach. Using this novel technology we were able to kill intracellular staphylococci in macrophages in vitro with gentamicin, and to rescue zebrafish embryos from lethal Staphylococcus aureus infection.[3] The way that this technology will help prevent resistance development around implants will be discussed.

In the Horizon 2020 ITN training network we aim to develop technology to produce antimicrobial medical devices by additive manufacturing ("3D-printing"), with in vitro antimicrobial activity and in vivo efficacy to prevent or treat infection. Design of the program and initial results will be presented.

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New Anti-Biofilm Strategies from Understanding Biofilm Life Cycle Dynamics

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Understanding the mechanisms of biofilm development and dispersal is providing new ways to manage biofilms and overcome their tolerance towards antimicrobial compounds. While most of these discoveries have been made in the context of single-species biofilms, an additional major outstanding challenge is to move beyond single-species models into controls for multispecies communities' characteristic of real-world settings or infections. In this presentation, I will review our research in biofilm control, including our clinical translation of new antibiofilm technology, in this case to disrupt biofilms within respiratory infections within Cystic Fibrosis in human clinical trials.

As part of this presentation I will also provide an overview of the recently awarded National Biofilms Innovation Centre, funded by BBSRC, Innovate UK, plus Industry and academic partners, and discuss the technologies, funding and collaborative opportunities that NBIC provides.