RiHN
Redistributed Manufacturing in Healthcare Network

EPSRC Redistributed Manufacturing in Deployed Medical Care

Funding Application Form

1. RESEARCH DETAILS					
	PROJECT TITLE:	AMPlify: redistributed manufacturing of antimicrobial peptides for wound care			
	RESEARCH FUNDING AREA:	Additive Manufacturing	☐	Cell and Tissue Manufacturing	☐
		Clinical Fluids Manufacturing	☐	Pharmaceutical Manufacturing	✔
		Cross Disciplinary [please state]:			☐
	LEAD INSTITUTION:	**University of Cambridge**			
	PRINCIPLE INVESTIGATOR:	**Dr Jennifer Molloy/Prof Lisa Hall**			
	PROPOSED DURATIO 1\N:	**Six months**			
	FUNDING:	£ 85,777 @100% FEC Total Project Cost			
		£ 68,622 @80% FEC RIHN		£ 17,155 @20% FEC Host Institute(s)	
	COLLABORATOR CONTRIBUTION(S):	£ 3000			
	SUMMARY (max. 250 Words):				

	Traumatic injuries are at high risk of infections by the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp*.) which require rapid diagnosis and treatment before they become established and put the patient at risk of debilitating chronic infections and complications such as tissue damage, amputation and sepsis. In the first days after an injury, complications due to such wound infections are one of the most common causes of death. Unfortunately, ESKAPE pathogens are often resistant to frontline antibiotics, making treatment more challenging and requiring specialist pharmaceuticals which can be difficult to access in remote areas, conflict zones or in the midst of humanitarian crises. To address this challenge, we will prototype a lyophilized reagent to produce antimicrobial peptides (AMPs) on-demand, off-grid and without a cold-chain. AMPs have shown significant promise in wound treatment and are less susceptible to the evolution of resistance than small molecule antibiotics. We will increase AMP stability and enhance their therapeutic properties by immobilisation on a chitosan hydrogel using auto-assembling protein interactions, creating a preparation that is designed for deployment as a frontline treatment in emergency care. AMPlify is based on a Cell-Free Protein Synthesis (CFPS) formulation which is highly flexible and can be "reprogrammed" with DNA to manufacture new and improved AMPs or other therapeutic molecules. CFPS can also be used as a biosensing platform, offering future opportunities to develop a theranostic system that diagnoses specific ESKAPE pathogens then uses genetic logic circuits to activate manufacturing of the appropriate AMP or therapeutic.

2. RESEARCH BACKGROUND (max. 300 Words)

Trauma to the extremities is a common injury for both civilians and military personnel in conflict and humanitarian crisis zones. Wound infections are also common in these environments. For example, 10-20% of the nearly 6000 casualties who sustained gunshot wounds in the Gaza Mass Demonstrations 2018-19 went on to develop osteomyelitis[1] and during military operations in Iraq and Afghanistan, infectious complications were reported in >25% of those evacuated for trauma (Yun et al., 2016).

Infections lead to poor outcomes including additional surgery and longer stays in hospital (Älgå et al., 2018) so rapid diagnosis and treatment is critical. However, this relies on a time-consuming culture of microbes or alternative diagnostics that require expensive equipment, a cold-chain and trained personnel. Even once diagnosed, many of the most problematic pathogens such as *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp* (grouped as ESKAPE) are resistant to antibiotics and therefore challenging to treat. Some studies have suggested that up to 73% of patients with infection in conflict settings have multidrug-resistant bacteria (Älgå et al., 2018).

Cell-free protein synthesis (CFPS) technology has been transformed over the last decade as a distributed manufacturing technology for diagnostics, therapeutics and vaccines that avoids cold-chain distribution and offers latent capability for scaling up manufacturing during emergencies (Pardee et al., 2016a & 2016b; Stark et al., 2019, Melinek at al., 2020). We propose that redistributed manufacturing (RDM) systems using CFPS could eventually be deployed to deliver rapid diagnosis and production of therapeutics in crisis zones. Basic principles have been demonstrated but key research gaps include i) improving CFPS production using

[1] http://www.emro.who.int/images/stories/palestine/documents/who_emergency_trauma_response_to_gaza_demonstrations_2018_2019.pdf?ua=1&ua=1&ua=1

basic equipment with a focus on scalability, stability and resource minimisation; ii) refining specific applications of CFPS in collaboration with stakeholders; iii) leveraging biological processes to bypass the need for downstream processing of therapeutics.

2. AIM AND OBJECTIVES (max. 100 Words)

We will develop AMPlify, a proof-of principle of a cold-chain-free biological RDM system that is stable for 3 months at 40C and can manufacture single-doses (2 ug/ml) of the antimicrobial peptide (AMP) RP557, which has shown promise for treatment of infected wounds (Woodburn et al., 2019). We will demonstrate production and immobilisation onto chitosan nanoparticles within six hours with high purity, investigate robustness of the RDM process and the potential impact of this research and the broader application of Cell-Free Biomanufacturing in Crisis Zones, in collaboration with clinicians, NGOs and international agencies.

3. NOVELTY AND TIMELINESS (max. 200 Words)

The rising prevalence of multidrug antimicrobial resistance (MDR) and recalcitrant biofilms is a major and urgent concern in field-based emergency trauma medicine. AMPlify introduces a novel CFPS as a platform that could both diagnose and manufacture an AMP therapeutic in a distributed manner and can be "reprogrammed" with new DNA in response to changing resistance phenotypes or improved AMPs. This would offer a flexible and modular distributed biomanufacturing system that could be repurposed for other medical applications.

AMPlify will focus on manufacturing antimicrobial peptides, which has been demonstrated in "fresh" cell-free (e.g. Borrero et al., 2019) and simultaneously binding the AMPs to chitosan as a combined purification and drug delivery mechanism, reducing the complexity of downstream processing. Our application builds on foundational conducted across several disciplines only in the last two years and has thus not been previously addressed.

The next phase of research would be to develop a multiplex cell-free biosensor for the ESKAPE pathogens and a genetic logic circuit to tie detection to manufacturing of appropriate AMPs, generating a novel closed system that merges two major features of CFPS technology in new ways and would be fundable in several contexts and calls.

4. METHODOLOGY (max. 750 Words)

Work Package 1: Improved formulation of low-cost cell-free protein synthesis reagents [Lead: Dr Nicole Prandi]

We developed a CFPS from crude E. coli extract that can act as a "universal reagent" for on-demand production of specialty enzymes and proteins, is 25% cheaper than the current formulations and stable for several months at 40 C. We will now apply our lyophilization methodology to a "pure" cell-free extract system generated by co-culture of CFPS components (Lavickova & Maerkl, 2019). Pure CFPS is more controllable, adaptable and better suited to express AMP therapeutics.

Work Package 2: Expression of AMP RP557 in a CFPS [Lead: Dr Nicole Prandi]

RP557 is a recent, promising synthetic AMP that demonstrated potent wound healing in lab tests (Woodburn et al., 2019). To express RP557 we need to modify the CFPS to form disulphide bridges by using glutathione buffer and expressing disulfide isomerase (Matsuda et al., 2013).

Work Package 3: Immobilisation of RP557 to chitosan nanoparticles for purification [Lead: Dr Nicole Prandi]

AMPs have shortcomings: they degrade rapidly and display toxicity to mammalian cells. Binding AMPs to polymers has been shown to improve their stability and chitosan is an attractive choice, already being biocompatible, antimicrobial and wound-healing. Chitosan-AMP conjugates such as Dhvar-5 (immobilized via “click” chemistry) have already shown promise as antimicrobial coatings for medical implants.

To remove downstream chemical processing, we will fuse RP557 with a chitosan binding domain protein tag (CBD), enabling immobilisation on chitosan nanoparticles during expression in CFPS. We will test the stability of the Chitosan-CBD interaction and also AMP activity including minimal inhibitory concentration of RP557 and RP557-chitosan nanoparticles on *E. coli, B. subtilis, and S. aureus MRSA* biofilms via Etest and time-kill test.

Work Package 4: Stakeholder engagement and feasibility assessment [Lead: Dr Jenny Molloy]

We will host a two-hour, virtual workshop on challenges and opportunities for Cell-Free Biomanufacturing in Crisis Zones, bringing together ~15 professionals inc regulators (WHO), humanitarian organisations (MSF, UNDP), NGOs (Glia, Field Ready), companies and world-leading CFPS researchers (e.g. Mike Jewett, Keith Pardee). We are actively collaborating with these groups scientifically or through policy initiatives such as the UN Technology Access Partnership (TAP); with whom the PIs are developing a roadmap for local production of diagnostics in LMICs.

Following the workshop, we will undertake further discussions and develop a collaborative report assessing the feasibility of deploying RDM solutions such as AMPlify and prioritising future research questions. Report release will be followed by a one-hour webinar and Q&A.

Contribution of the Research Team

The experimental research will be undertaken by Dr Nicole Prandi (100% FTE), managed by PI Dr Jenny Molloy (10% FTE) who will lead on stakeholder engagement activities. The project will be supervised by Prof Lisa Hall as senior PI.

Our partners Glia and Field Ready will provide valuable insights into the application and context of AMPlify and guide the direction of the future research to better meet the needs of emergency and trauma medics in crisis zones.

Objectives and Milestones

WP	***Objective Milestone***	***Description***	***Month***
1	O1.1	Active pure CFPS system that is stable following lyophilization for at least 2 weeks at ambient temperature	M2
	MS2	**Active pure CFPS that is stable for at least three months at 40 C**	**M4**
2	O2.1	Adaptation of cell-free extract to expression of proteins with disulphide bridges	M1
	O2.2	Expression of active RP557 demonstrating antimicrobial activity.	M2
	MS1	**Demonstration of expression of single-dose levels (2 ug/ml)**	**M3**
3	O3.1	Cloning of peptide linkers and affinity tags with RP557	M3
	O3.2	Immobilisation of RP557 on chitosan nanoparticles	M4
	MS3	**Demonstration of one-step expression and immobilisation of single dose AMP in < 6 hours**	**M5**
4	O4.1	Virtual workshop on Cell-Free Biomanufacturing in Crisis Zones	M4
	O4.2		M5

	O4.3 **MS4**	Understanding feasibility and impact of bio-RDM in crisis zones through direct engagement with stakeholders Communication to external stakeholders and wider public through a web page and digestible blogs and social media engagement. **Publication of report from workshop and stakeholder engagement**	M6 **M6**

Project Management and Gantt Chart

Month/ Work Package	M1	M2	M3	M4	M5	M6
WP1		O1.1		MS2		
WP2	O2.1	O2.2	MS1			
WP3			O3.1	O3.2	MS3	
WP4				O4.1	O4.2	O4.3 | MS4

Project update meetings between the University of Cambridge team will take place weekly to ensure agile management of project progress and deliverables. Partners will be invited to formal project meetings every two months (including the workshop O4.1 in M4) and will be contacted via email with any queries and during report writing which will be a collaborative exercise.

5. PROJECT DELIVERABLES (max. 500 Words)

We will develop a range of outputs while developing a proof-of principle cold-chain-free RDM system for manufacturing single-dose levels of an antimicrobial peptide to promote wound healing.

Work Packages 1-3

1. *Proof-of principle of a cold-chain-free CFPS RDM system for manufacturing single-dose levels (2 ug/ml) of the antimicrobial peptide (AMP) RP557 immobilised onto chitosan, validated in the lab (TRL4).*
   This deliverable will comprise a package of DNA plasmids, protocols and datasets demonstrating the successful delivery of the main technical aim of the project. DNA plasmids will be made available to other researchers, protocols will be published via protocols.io and datasets will be shared via Zendodo and the University of Cambridge repsotiory.

2. *Manuscript for publication in a peer-reviewed journal detailing the development of NAME.*
   We will prepare an article for submission to a journal such as ACS Synthetic Biology to describe all technical and experimental work completed during the project, demonstrating a novel application of CFPS and the principles of biological RDM for crisis situations.

Work Package 4

1. *AMPlify web page and social media accounts*
   We will publish an AMPlify web page detailing the plans and partners. This will be updated with deliverables as they become available and at least three blog updates. Posts will also be circulated on social media including Twitter and LinkedIn to 500 followers by the end of the project.

2. *Two-hour virtual workshop on Cell-Free Biomanufacturing in Crisis Zones & follow up webinar*
   We will convene a short workshop as described in Q4, the deliverables will include a blog summary and photos to be posted to the AMPlify web page.

3. *Report from workshop and stakeholder engagement on opportunities and challenges for Cell-Free Biomanufacturing in Crisis Zones and future research priorities*.
   The Cell-Free Biomanufacturing in Crisis Zones Report will be published as a working paper on the AMPlify web page and the University fo Cambridge repository. If time allows, we will also prepare a commentary manuscript for publication.

Longer term outcomes and impact
The eventual impact emerging from successful research and further development of AMPlify will be agile clinical diagnosis and treatment for infected wounds in remote or deployed operational locations.

AMPlify is a six-month project which will provide a first-phase proof-of-principle of CFPS-based AMP production that is lab validated and can be taken forward to further research and development and to seed collaborations with end users, stakeholders and potential industry manufacturers.

6. PROFILE OF INVESTIGATORS (max. 100 Words per Investigator)

Prof Lisa Hall is head of the Cambridge Analytical Biotechnology group in the Department of Chemical Engineering and Biotechnology which focuses on understanding how biology can be interfaced with electronic, mechanical and optical systems and new ways to answer fundamental and applied questions concerning new measurement regimes. This links transduction technologies (electrochemistry, optics, ultrasound) with synthetic biology and nanomaterials to achieve sensors & diagnostic systems. Prof Hall has extensive experience of managing European and international collaborations, including diagnostics and field trials in Ghana, Malaysia and the Philippines to design locally produced POC nucleic acid diagnostics using synthetic biology techniques.

Dr Jenny Molloy is a Shuttleworth Fellow at the University of Cambridge, studying the role and impact of open approaches to intellectual property for a sustainable and equitable bioeconomy. Her research combines technical development using synthetic biology-based platform technologies with qualitative research on challenges faced by molecular biologists globally through interviews and case studies. She is currently chairing the Diagnostics Subgroup of the UN Technology Access Partnership to support local manufacturing of diagnostics and is Executive Director of a social enterprise establishing labs and research tool manufacturing in Africa.

Dr Nicole Prandi took a Masters in Industrial Biotechnology at the University of Padova with a research project on the engineering of a cyanobacterial strain for protein production. She continued her work in cyanobacteria with a 9 months internship in Imperial college London before starting her PhD in Synthetic biology at the University of Manchester on the microbial production of plant secondary metabolites. She is currently working at the University of Cambridge on automation of laboratory procedures and the development of a chloroplast-based protein expression system and has recently begun using cell-free production for low-cost protein expression.

7. JUSTIFICATION OF COSTING (max. 300 Words – *tables not included in word count*)

Dr Nicole Prandi will conduct the research over 6 months at 100% FTE, managed by Dr Jenny Molloy at 10% FTE .

We require reagents, consumables and DNA synthesis for which we have requested £15k based on previous experience with cell-free expression projects.

Data management will be undertaken by the project team at no extra cost and datasets will be deposited in Zenodo and Apollo, the University of Cambridge Data Repository under an open data license. Metadata in botb repositories is compliant with DataCite Metadata Schema minimum and persistence is guaranteed for beyond 5 years after the end of the study, complying with FAIR open data principles.

Our impact activities will not require further funds as the workshop will be held virtually. We will prepare the report in-house and host the web page and output on existing infrastructure including the lab group website and University of Cambridge repository.

Our project partners Glia and Field Ready will contribute their time in-kind to plan and attend the Cell-Free Biomanufacturing in Crisis Zones workshop and offer advice throughout the project and preparation of reports, as described in their letters of support.

Research Project Funding			
Summary Totals	**Indexed fEC Total**	**RiHN/EPSRC Contribution**	**Total to be shown on invoice**
Staff	28,725	22,980	22,980
Other	15,000	12,000	12,000
Estates (non-laboratory)	11,238	8,990	8,990
Indirect Costs	30,815	24,652	24,652
Total	**85,777.21**	**68,621.77**	**68,621.77**

Match Funding or Contribution In-Kind			
Summary Totals	**Indexed fEC Total**		**Total**
Glia In-Kind staff time - 1.5 days	£1500		£1500
Field Ready In-Kind staff time - 1.5 days	£1500		£1500
Total	**£3000**		**£3000**

8. DECLARATIONS (max. 200 Words)

Ethical standards: all parties will comply with University of Cambridge ethical processes and standards. Based on the current programme of work there is no requirement to obtain specific ethical approval but if project plans are changed e.g. stakeholder engagement proceeds with individual surveys rather than workshops, ethical approval will be sought due to the involvement of human subjects. We will remain

Data will be managed in line with EPSRC and University of Cambridge policy and good practices of FAIR open data sharing. Where possible datasets will be deposited in Zenodo and Apollo, the University of Cambridge repository under open data licences. Potential commericalisable intellectual property arising will be discussed with Cambridge Enterprise in line with University of Cambridge policy. We aim to release the results of this research as pre-competitive, open source outputs to promote further development of the platform technology.

REFERENCES (max. 10)

Älgå, Andreas, et al. "Infection with high proportion of multidrug-resistant bacteria in conflict-related injuries is associated with poor outcomes and excess resource consumption: a cohort study of Syrian patients treated in Jordan." BMC infectious diseases 18.1 (2018): 233.

Borrero, Juan, and Philippe Gabant. "PARAGEN 1.0: a standardized synthetic gene library for fast cell-free bacteriocin synthesis." Frontiers in bioengineering and biotechnology 7 (2019): 213.

Lavickova, Barbora, and Sebastian J. Maerkl. "A simple, robust, and low-cost method to produce the PURE cell-free system." ACS synthetic biology 8.2 (2019): 455-462.

Matsuda, Takayoshi, Satoru Watanabe, and Takanori Kigawa. "Cell-free synthesis system suitable for disulfide-containing proteins." Biochemical and Biophysical Research Communications 431.2 (2013): 296-301.

Melinek, Beatrice, et al. "Toward a Roadmap for Cell-Free Synthesis in Bioprocessing." Available from: https://bioprocessintl.com/upstream-processing/expression-platforms/toward-a-roadmap-for-cell-free-synthesis-in-bioprocessing/

Pardee, Keith, et al. "Portable, on-demand biomolecular manufacturing." Cell 167.1 (2016a): 248-259.

Pardee, Keith, et al. "Rapid, low-cost detection of Zika virus using programmable biomolecular components." Cell 165.5 (2016b): 1255-1266.

Stark, Jessica C., et al. "On-demand, cell-free biomanufacturing of conjugate vaccines at the point-of-care." BioRxiv (2019): 681841.

Woodburn, Kathryn Wynne, Jesse M. Jaynes, and L. Edward Clemens. "Evaluation of the antimicrobial peptide, RP557, for the broad-spectrum treatment of wound pathogens and biofilm." Frontiers in microbiology 10 (2019): 1688.

Yun, Heather C., et al. "Infection after orthopaedic trauma: prevention and treatment." Journal of orthopaedic trauma 30 (2016): S21-S26.