Easing the global burden of diarrhoeal disease: can synthetic biology help?

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Abstract The Millennium Declaration committed the 193 member states of the United Nations to end poverty by 2015. Despite the efforts of the UN and World Health Organisation, and the G8 commitment to spend a fixed proportion of gross national income on overseas aid, more than 2.6 billion people still lack access to proper sanitation. The absence of effective public health strategies in developing countries results in significant health burdens following gastrointestinal infections. Diarrhoea associated with infections resulting from oral-faecal contamination is the second leading cause of death in children under 5 years of age, primarily in Africa and South Asia. Currently there are no appropriate vaccines that could be easily administered on a global scale to prevent these infections. Synthetic biology has the potential to contribute to development of such vaccines. Our work is directed at developing a range of multivalent oral vaccines against the most common diarrhoea-causing bacteria, e.g., *Escherichia coli*, *Shigella* and *Salmonella*. If synthetic biology is to avoid the suspicion and possible revulsion of the public, scientists need to demonstrate that this new field has something real to offer.

Keywords Global health · Diarrhoeal disease · Synthetic biology · Vaccines

Diarrhoeal diseases in developing countries

The United Nations (UN) Millennium Declaration committed the countries of the world to end poverty by 2015. One of the overarching themes for the project is to eradicate disease and promote health. Despite the efforts of the UN and World Health Organisation (WHO), more than 2.6 billion people still lack access to proper sanitation (WHO report 2011). The absence of effective public health strategies in developing countries results in significant health burdens following gastrointestinal (GI) infections caused by viral, bacterial and parasitic pathogens. Diarrhoea associated with infections resulting from oral-faecal contamination is the second leading cause of death in children under 5 years of age. Children living in Africa and Asia are most at risk from diarrhoea. It is estimated that more than 2 billion children under five contract diarrhoea and that 1.5 million of them die as a result (WHO 2009). Children are more prone to these infections, and the associated complications, because they often suffer from poor nutrition and impaired immune status. While basic strategies such as hand washing can reduce infection rates, the absence of sanitation, and the associated lack of clean drinking water, means that children will continue to die.

Why should we care?

The Millennium Development Goals (MDG) define the right for all people to have access to education, medical treatment and be free from disease (Annan 2000). There is a moral obligation to ensure that the MDG are met, however, in times of economic instability it may be hard to convince the electorate that international development is a priority. At a practical level, health inequality in

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economically deprived countries may drive mass migration, political unrest, food security issues and even terrorism. This implies there should be significant motivation at governmental level to drive foreign and security policies to ensure economic and medical intervention. Despite the impetus of the Gleneagles G8 summit in 2005, most governments have failed to meet their commitment to spend 0.7 % of gross national income on overseas aid. While this is understandable in view of the current global economic climate, the gap in funding for solutions to neglected diseases is, by necessity, being addressed by philanthropic organisations such as the Bill and Melinda Gates Foundation and the Global Fund to Fight Aids, Tuberculosis and Malaria. The Gates Foundation is driven by the tenet that “all lives have equal value”, however, they acknowledge that “philanthropy plays an important but limited role” (www.gatesfoundation.org/about/Pages/guiding-principles.aspx; see Rooke, this issue). The Foundation also realises the potential for groundbreaking technologies to change the plight of millions of people, as demonstrated by their call for proposals to tackle global health issues based on emerging synthetic biology approaches. Ultimately, however, it is the responsibility of governments in developed countries to ensure the MDG are met.

Solutions to the problem

The infrastructure required to provide basic sanitation, particularly in rural areas, is outwith the financial constraints of many developing countries and will require decades of economic growth, external investment and construction. A more rapid solution is required, with immunization being a potential route to control or prevent diarrhoeal disease. The WHO has recommended that a global rotavirus vaccination programme is initiated to eradicate the primary cause of childhood diarrhoea. A number of bacterial pathogens also contribute significantly to deaths resulting from diarrhoea, these include *Escherichia coli*, *Shigella*, *Campylobacter* and *Salmonella*; with *Vibrio cholerae* having a significant impact following natural disasters. Currently there are no appropriate vaccines that could be used easily on a global scale to prevent these infections. Synthetic biology has the potential to contribute to development of such vaccines.

Evolution of synthetic biology

The Royal Society defined synthetic biology as “the design and construction of novel artificial biological pathways, organisms or devices, or the redesign of existing natural biological systems”. How does this differ to “traditional”

molecular biology? Since the 1970s, recombinant technology has not only been used to interrogate biological systems in order to further our understanding, but has also led a revolution in biotechnology. Expression in *E. coli* of the recombinant subunit vaccine against hepatitis B could be described as one of the first examples of a biomedically important artificial organism (Murray et al. 1984). Production of chimeric proteins in both prokaryotic and eukaryotic cells is not new and could be described as redesigning natural biological systems. The promise of synthetic biology comes not from the piecemeal alterations of previous methodologies, but from the genomic scale of possible changes and the ability to use the established genetic code to design novel systems not previously seen in nature. Synthetic biology borrows from, and builds on, many scientific traditions including genomics, molecular biology, bioinformatics, chemistry and the discipline of engineering. As such it is an evolutionary step in biology and care should be given to the promise of revolutionary solutions. The public have been sold the ideals of personalized medicine arising from the human genome project, and while genetic testing has flourished, little in terms of groundbreaking treatments has emerged (Veenstra et al. 2010). Indeed, the ethical issues surrounding genetic testing have generated further dilemmas within the healthcare industry (Martin et al. 2010). Synthetic biology needs to avoid a similar scenario.

A synthetic biology-based approach to vaccines against diarrhoea

Synthetic biology has the potential to influence various aspects of global health. Engineered biomolecules, synthetic gene networks and programmable organisms can be used for drug development, detection of environmental contaminants and regenerative medicine (Burbelo et al. 2010; Ruder et al. 2011; French et al. 2011; see Carothers, this issue). Cheaper drug production is a core application, with the engineering of yeast to produce the precursor for an anti-malarial drug the flagship achievement (Ro et al. 2006). Microbial manipulation is not the only target, with human genetic systems being synthesized to enable identification of novel drugs for treatment of globally important diseases, such as multidrug-resistant tuberculosis (Weber et al. 2008). The indirect eradication of disease is also possible, with mosquitoes being modified to limit the spread of malaria (Windbichler et al. 2011).

Our work uses synthetic biology to develop a range of multivalent oral vaccines against the most common diarrhoea-causing bacteria, e.g., *E. coli*, *Shigella* and *Salmonella*. The primary method currently used for delivering vaccines in the industrialised west is injection. The use of needles limits accessibility of vaccines to the populations

of developing countries, particularly because of costs associated with equipment and trained medical staff. A paradigm shift is required in the administration of vaccines if large rural populations are to be protected against common diarrhoeal diseases caused by bacteria. The use of stable oral vaccines would reduce the need for highly trained staff and could remove the requirement for a cold storage chain. Mass administration of the oral polio vaccine is a powerful example of how immunization can control and eradicate disease in both developed and developing countries.

Vaccines against pathogenic bacteria or viruses generally use live-attenuated strains or killed cells, such as vaccines against polio, measles and tuberculosis. Since vaccines will only protect against pathogens with the same epitopes, multiple strains need to be propagated to provide immunological coverage. The use of live vaccines also carries the risk of causing infection in immuno-compromised patients.

An oral vaccine needs to elicit a secreted IgA response for mucosal immunity and IgG-mediated humoral immunity. Mucosal immunity will generally be directed against surface molecules on bacterial pathogens, so the antigens for the vaccine need to be either outer membrane proteins (for Gram-negative bacteria) and/or polysaccharides. We are designing and building synthetic operons that allow expression of multiple antigens in a heterologous bacterial host. Ultimately, we plan to purify and separate antigens from live organisms, and administer them orally on nanoparticles as a vaccine. Such antigenic particles, when released in the GI tract, will be engulfed by dendritic cells and the antigens subsequently presented to CD4⁺ T cells. This delivery route is preferred because it avoids the use of live, genetically modified organisms as the vaccine and so prevents potentially adverse pathological side-effects.

Host organism (chassis) choice and construction

To facilitate downstream processing of antigens, we have specific requirements for the expression organism or chassis. The bacterium of choice needs to have low endotoxicity, be capable of extensive polysaccharide biosynthesis and be able to grow in a simple inorganic salt medium. Most synthetic biology projects utilize *E. coli* or a similar model organism as the chassis, with the vast majority of controlling components, e.g., promoters, in the BioBricks repository being based on sequences derived from *E. coli* (<http://partsregistry.org/Catalog>). This bacterium, however, is not the most suitable for purifying surface expressed antigens because the lipopolysaccharide (LPS) in the outer membrane is a potent inducer of the pro-inflammatory response (Hoshino et al. 1999). LPS

contamination of any vaccine would therefore have undesirable side-effects and its removal would add extra costs to production. We need to look beyond the traditional bacterial hosts favored by molecular biology and exploit more diverse prokaryotes. For example, the human GI tract contains more than 1,500 bacterial species that contribute to health and development, and many of the resident Gram-negative bacteria synthesize different forms of LPS that do not have high endotoxicity. We are currently developing synthetic approaches for other commensal bacteria, such as members of the genera *Bacteroides* and *Clostridium*, that are genetically very different to *E. coli*, but which fulfill the requirements for easier product recovery. The disadvantages of working with less-studied bacteria are that regulatory sequences are less well defined and there are significant barriers to introduction of foreign DNA due to multiple resident restriction-modification (R-M) systems (Blakely and Murray 2009). Utilizing different bacteria for synthetic engineering has been made possible by the ease of genome sequencing and genomic technologies, such as transcriptomics, because we can now define transcription start sites and identify novel promoters.

Driving public opinion: and public safety

If synthetic biology is to avoid the suspicion and possible revulsion of the public, similar to that experienced with genetically modified crops in Europe, scientists need to demonstrate that this new field has something real to offer. Funding bodies often make public engagement a requirement for award of grants, but many scientists feel unprepared to make that connection. A dialogue between scientists and tax payers needs to exist so the public can be informed of the technology but in the absence of “hype”. The same examples of synthetic biology are repeatedly used to emphasize the benefits, e.g., artemisinin, but new tangible impacts are scarce. Public acceptance would be better served if a laudable goal, such as global health equality, was the key metric and using synthetic biology to achieve that goal was merely a tool. Philosophical discussions about intangible subjects, such as defining when an engineered organism becomes a new life-form, provide little public focus for a progressive science. How far do we need to take public consultation, particularly with final recipients of any product (see Betten et al., this issue)? Do we require moral consent from the citizens of developing countries to develop and distribute synthetic biology-based vaccines? How do we explain potential ethical dilemmas to a population that may have limited access to education? Do we treat synthetic biology products the same as any other pharmaceutical? Is the source irrelevant and the purpose more important?

The biggest public concerns regarding synthetic biology research are biosafety and biosecurity. Given that evermore complex novel biological systems will be produced, what are scientists doing to ensure environmental and human safety? It can be argued that synthetic biology does not raise any new concerns that traditional genetic engineering has not already addressed. There are regulations in place for research involving genetically modified organisms. In Europe, there is institutional governance of the contained use of genetically modified organisms, as directed by the European Commission (Directive 2009/41/EC). However, one of the potential problems with synthetic biology is the interdisciplinary nature of the field. Biologists, by virtue of their training and background, have the conceptual framework for understanding biological outcomes and effects such as pathogenesis or environmental contamination, but synthetic biology also involves engineers, mathematicians and computer programmers (Garfinkel et al. 2007; Schmidt et al. 2009). Involving other disciplines in laboratory work with microorganisms has potential pitfalls if not adequately managed. Therefore, it is essential for research institutions to provide a framework within which there is adequate training and knowledge-exchange between researchers from different fields (Bailey et al. 2012; Schmidt et al. 2009). Further, it is imperative that technical precautions are taken to generate organisms that have intrinsic “Bio-firewalls”, so not only will they have reduced viability in the natural environment but the engineered DNA will be prevented from being acquired and transmitted by horizontal gene transfer. Recently, inducible synthetic lysis devices under the control of external signals, such as temperature, have been engineered to ensure the destruction of the modified bacterium on entry into the host (Pasotti et al. 2011). This protection of natural diversity will become even more important when industrial scale processes are implemented.

For our own work, appropriate biosafety conditions need to prevent these synthetic derivatives from potentially colonizing the GI tract of laboratory workers. We can meet containment criteria by generating auxotrophic strains, which require essential nutrients to be artificially provided, and by deleting the resident R-M systems, which would make the bacterium extremely sensitive to their natural predators, bacteriophages, which are abundant in the GI tract. The strategy we aim to employ for vaccination uses non-infectious nanoparticles, so does not require delivery of the modified bacterium to the human GI tract.

Governance and legislation

The governance of synthetic biology requires mandatory rules for the production and distribution of certain products

within the research environment, as seen for genetically modified organisms and products in Europe, but regulation must not stifle science (Kelle 2007; Presidential Commission 2010). Flexibility of rules is required when considering different situations, such as a contained laboratory experiment compared to release of a synthetic organism into the environment (Kelle 2007). Governance of such a vast and varied field is difficult and requires increased awareness of researchers as well as involvement by leading scientists in the field rather than just policymakers (European Commission 2010).

For most researchers, self-governance appears to be most appropriate (Maurer et al. 2006). While there should be institutional and governmental support for a culture of self-regulation, and responsibility within the research community, there must also be institutional monitoring and enforcement of existing regulations (Presidential Commission 2010). Institutional biosafety committees and funding bodies must evaluate the risks of research and ensure compliance with national and international guidelines, but should also alter regulations when necessary (Balmer and Martin 2008; Garfinkel et al. 2007). The monitoring of compliance must be supported by laws and extend beyond researchers to manufacturers of products such as synthetic genes (Church 2005). There should be screening of all orders placed with gene synthesis companies and of the people placing the orders, and this monitoring should extend to multiple orders as well to avoid dangerous sequences being assembled in smaller pieces (Garfinkel et al. 2007; Maurer et al. 2006; Schmidt et al. 2009). There is also a need for universal databases that include a list of all parent strains and genes with potential for harm through which ordered sequences, precursor chemicals and designer cells could be cross-checked (Church 2005; Schmidt et al. 2009). For such monitoring to be successful, international harmonization of standards and registries is crucial (van Est et al. 2007; European Commission 2010; UK synthetic biology roadmap 2012).

Currently in the UK, synthetic biology is covered by the same regulations as those for genetically modified organisms (Bailey et al. 2012). The Health and Safety Executive states that risk assessment for synthetic biology should “acknowledge uncertainty and deal with it using the precautionary principle” (Bradbrook 2007). The potential global impact of biology derived problems is illustrated by the furore over the laboratory development of a more transmissible form of the H5N1 strain of influenza, which has the notional ability to generate a lethal pandemic if accidentally released from the lab (Fouchier et al. 2012). This illustrates the importance of biosafety and the possibility that biologists can under-appreciate both public and governmental concerns over biocontainment. While this is a worst-case-scenario, the impacts of local actions with

regard to synthetic biology, whether relating to personal health or environmental release, need to be addressed with rigor. At the same time, unnecessary regulations must not hamper progress. It would be prudent for scientists and regulators, such as the Health and Safety Executive and the Home Office in the UK, to work together and regularly review the legislation as the field evolves. This would ensure that appropriate safeguards are maintained and should ensure positive public opinion, and subsequent funding, for a field with promising solutions for the future. Systems such as the HSE's Horizon Scanning are therefore essential to safeguard biosafety and biosecurity of synthetic biology research (Bradbrook 2007).

Developing and exploiting potential products

As with any scientific endeavour, one of the limitations for synthetic biology-driven research to solve global health problems is funding. Many national agencies have voiced an interest in providing resources for synthetic biology, but little seems to have materialized in terms of global health. For example, of the five projects included in the European Commission framework programme 7 EuroSYNBIO consortium, only one had a biomedical interest. The direction of science funding is generally towards improving national economic performance and not solving global health problems associated with bacteria, possibly with the exception of research on *Mycobacterium tuberculosis*. The future of European funding for research on solutions to global health issues is currently unclear and will depend on how the "Horizon 2020" financial implement is wielded by the EU. If the international community is serious about implementing the MDG, policy-makers need to move beyond rhetoric and provide "ring-fenced" funding for directed research to solve major global medical problems.

Assuming that we can make novel therapies, based on synthetic biology, that could treat or prevent millions of deaths, how do we ensure that such technology is distributed to countries dominated by poverty (see Hollis, this issue)? Most academic institutions where research is undertaken have policies regarding commercialisation of inventions. The accepted route for protection of a technology, especially with a large potential for impact, is by application for international patents. Other routes involve granting of non-exclusive licenses or "open technology" access where no financial charge is levied. The morally acceptable approach for a product that has global ramifications might appear to be through a non-licensing donation. Many supporters of synthetic biology have likened this aspect of the field to open access computer software. The institutional argument for patenting a new technology, however, is that in the current commercially driven world

the only way to develop a product is by attracting industrial investment. The moral dilemma is therefore not whether to patent, but rather which company will partner a project and what are their ethical principles (see Douglas and Stermerding, this issue). Individual scientists are often not able to judge the merits of a company, so it is the responsibility of employing institutions to develop policies for ensuring ethical partnerships.

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References

- Annan KA (2000) <http://www.un.org/millennium/sg/report/full.htm>
- Bailey C, Metcalf H, Crook B (2012) Synthetic biology: A review of the technology, and current and future needs from the regulatory framework in Great Britain. Health and Safety Executive, Research Report RR944. <http://www.hse.gov.uk/research/rtrpdf/rr944.pdf>
- Balmer A, Martin P (2008) Synthetic biology: social and ethical challenges institute for science and society, University of Nottingham. <http://www.bbsrc.ac.uk/organisation/policies/reviews/scientific-areas/0806-synthetic-biology.aspx>
- Blakely GW, Murray NE (2009) DNA restriction and modification, in the encyclopedia of microbiology, 3rd Edition, edited by Moselio Schaechter, Elsevier
- Bradbrook S (2007) HSE horizon scanning intelligence group short report synthetic biology. SR010. <http://www.hse.gov.uk/horizons/>
- Burbelo PD, Ching KH, Han BL, Klimavicz CM, Iadarola MJ (2010) Synthetic biology for translational research. *Am J Trans Res* 2:381–389
- Church G (2005) Let us go forth and safely multiply. *Nature* 438:423. doi:10.1038/438423a
- Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. Off J Eur Union. L 125/75. http://www.bmwf.gv.at/fileadmin/user_upload/forschung/gentechnik/2009-41-EC.pdf
- European Commission (2010) Synthetic Biology: From Science to Governance. A workshop organised by the European Commission's Directorate-General for Health and Consumers, Brussels. http://ec.europa.eu/health/.../docs/synbio_workshop_report_en.pdf
- Fouchier R, Osterhaus A, Steinbruner J et al (2012) Preventing pandemics: the fight over flu. *Nature* 481:257–259. doi:10.1038/481257a
- French CE, de Mora K, Joshi N, Ellick A, Haseloff J, Ajioka J (2011) Synthetic biology And the art of biosensor design. In: Institute of Medicine (US) Forum on Microbial Threats. The Science and Applications of Synthetic and Systems Biology: Workshop Summary. Washington (DC): National Academies Press (US). A5. <http://www.ncbi.nlm.nih.gov/books/NBK84465/>
- Garfinkel MS, Endy D, Epstein GL, Friedman RM (2007) Synthetic genomics: options for governance available here. <http://www.jcvi.org/cms/fileadmin/site/research/projects/synthetic-genomics-report/synthetic-genomics-report.pdf>
- Hoshino K et al (1999) Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the *Lps* gene product. *J Immunol* 162:3749–3752
- ISBN: 978-92-4-159841-5 (NLM classification: WS 312) (WHO). http://whqlibdoc.who.int/publications/2009/9789241598415_eng.pdf

- Kelle A (2007) Synthetic biology and biosecurity: awareness in Europe. Bradford Science and Technology Report No.9. http://www.synbiosafe.eu/uploads///pdf/Synbiosafe-Biosecurity_awareness_in_Europe_Kelle.pdf
- Martin DK, Greenwood HL, Nisker J (2010) Public perceptions of ethical issues regarding adult predictive genetic testing. *Health Care Anal* 18:103–112
- Maurer SM, Lucas KV, Terrell S (2006) From understanding to action: community-based options for improving safety and security in synthetic biology. Goldman School of Public Policy, University of California at Berkeley. <http://gspp.berkeley.edu/iths/UC%20White%20Paper.pdf>
- Murray K, Bruce SA, Hinnen A, Wingfield P, van Erd PM, de Reus A, Schellekens H (1984) Hepatitis B virus antigens made in microbial cells immunise against viral infection. *EMBO J* 3:645–650
- Pasotti L, Zucca S, Lupotto M, Cusella De Angelis MG, Magni P (2011) Characterization of a synthetic bacterial self-destruction device for programmed cell death and for recombinant proteins release. *J Biol Eng* 5
- Presidential Commission for the Study of Bioethical Issues (2010). New directions: the ethics of synthetic biology and emerging technologies. http://bioethics.gov/cms/sites/default/files/PCSBISynthetic-Biology-Report-12.16.10_0.pdf
- WHO report (2011) The world health report—Health systems financing: the path to universal coverage. <http://www.who.int/whr/2010/en/index.html>
- Ro D, Paradise EM, Ouellet M, Fisher KJ, Newman KL, Ndungu JM, Ho KA, Eachus RA, Ham TS, Kirby J, Chang MCY, Withers ST, Shiba Y, Sarpong R, Keasling JD (2006) Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* 440:940–943
- Ruder WC, Lu T, Collins JJ (2011) Synthetic biology moving into the clinic. *Science* 333:1248–1252
- Schmidt M, Ganguli-Mitra A, Torgersen H, Kelle A, Deplanes A, Biller-Andorno N (2009) A priority paper for the societal and ethical aspects of synthetic biology. *Syst Synth Biol* 3:3–7
- Technology Strategy Board on behalf of UK Synthetic Biology Roadmap Coordination Group (2012). A synthetic biology roadmap for the UK. <http://www.rcuk.ac.uk/Publications/reports/Pages/syntheticbiologyroadmap.aspx>
- van Est R, de Vriend H, Walhout B (2007) Constructing Life: The World of Synthetic Biology, Rathenau Institute—Technology Assessment. [http://www.synbiosafe.eu/uploads///pdf/BAP_Synthetic_biology_nov2007\[1\].pdf](http://www.synbiosafe.eu/uploads///pdf/BAP_Synthetic_biology_nov2007[1].pdf)
- Veenstra DL, Roth JA, Garrison LP Jr, Ramsey SD, Burke W (2010) A formal risk-benefit framework for genomic tests: facilitating the appropriate translation of genomics into clinical practice. *Genetics in Medicine* 12:686–693. doi:10.1097/GIM.0b013e3181eff533
- Weber W, Schoenmakers R, Keller B, Gitzinger M, Grau T, Daoud-El Baba M, Sander P, Fussenegger M (2008) A synthetic mammalian gene circuit reveals antituberculosis compounds. *PNAS* 105:9994–9998
- WHO (2009) Diarrhoea: Why children are still dying and what can be done
- Windbichler N, Menichelli M, Papathanos PA, Thyme SB, Li H, Ulge UY, Hovde BT, Baker D, Monnat RJ Jr, Burt A, Crisanti A (2011) A synthetic homing endonuclease-based gene drive system in the human malaria mosquito. *Nature* 473:212–217