Microbial synthetic biology for human therapeutics

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Abstract The emerging field of synthetic biology holds tremendous potential for developing novel drugs to treat various human conditions. The current study discusses the scope of synthetic biology for human therapeutics via microbial approach. In this context, synthetic biology aims at designing, engineering and building new microbial synthetic cells that do not pre-exist in nature as well as re-engineer existing microbes for synthesis of therapeutic products. It is expected that the construction of novel microbial genetic circuitry for human therapeutics will greatly benefit from the data generated by ‘omics’ approaches and multidisciplinary nature of synthetic biology. Development of novel antimicrobial drugs and vaccines by engineering microbial systems are a promising area of research in the field of synthetic biology for human theragnostics. Expression of plant based medicinal compounds in the microbial system using synthetic biology tools is another avenue dealt in the present study. Additionally, the study suggest that the traditional medicinal knowledge can do value addition for developing novel drugs in the microbial systems using synthetic biology tools. The presented work envisions the success of synthetic biology for human therapeutics via microbial approach in a holistic manner. Keeping this in view, various legal and socio-ethical concerns emerging from the use of synthetic biology via microbial approach such as patenting, biosafety and biosecurity issues have been touched upon in the later sections.

Keywords Antibiotics · Biosafety · Diseases · Intellectual property · Metabolic engineering · Secondary metabolites · Traditional knowledge

Abbreviations

SAVE Synthetic attenuated virus engineering
NCDs Non-communicable diseases
IP Intellectual property
FDA Food and drug administration
DNA Deoxyribonucleic acid

Introduction

Synthetic biology can be defined as a combinatorial approach where the basic elements, governing the operational functionality of a living cell, are placed together to generate life forms with novel characteristics and properties (Saukshmya and Chugh 2010). The term synthetic biology was first introduced by the Polish geneticist Wacław Szybalski in the year 1974, however, it gained popularity and usage in the mainstream science in the year 2004 when the first international meeting on synthetic biology was held at MIT, USA (Szybalski 1974; Tucker and Zilinkas 2006).

Synthetic biology can be considered as extreme genetic engineering as it is an amalgamation of the principles of engineering and biology that provide a new paradigm for generation of novel applications in diverse areas such as therapeutics, bioremediation, waste resource management and biofuel production (Savage et al. 2008; Schmidt 2010; Purnick and Weiss 2009; Brenner and Arnold 2011). It is a multidisciplinary science in a true sense as it derives knowledge from various disciplines such as genetics,

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nanotechnology, chemical engineering, robotics, systems biology and computational biology, in order to manipulate living cells to obtain desired functions. It is speculated that with further technological advancements and understanding of the biological phenomenon, greater avenues for synthetic biology applications will emerge which would allow incorporation of the information generated through various projects based on genomics, proteomics, metabolomics and phenomics. Synthetic biology aims at enablement of “creation” of living organisms with user-defined genetic circuitry and novel characteristics by modulating, regulating and redesigning the gene expression profile of the host organism, enabling its exploitation for various applications (Marguet et al. 2007). As a stepping stone towards this direction, micro-organisms (such as *Escherichia coli* and *Streptomyces*) with minimal genome that is with genes essential for their survival have been created (Sung et al. 2009; Komatsu et al. 2010). Such microbes can be further transformed with genes conferring them novel functions. The current research in synthetic biology focuses on developing microorganisms suitable for application in therapeutics, biofuel production and bioremediation (Fig. 1). The present study focuses on applications of synthetic biology in human therapeutics using microbial system based approach.

Diseases belonging to various categories such as communicable, non-communicable and now, lifestyle related disorders have plagued human civilization from time to time. According to a World Health Organization (WHO) report, out of ten deaths occurring globally, six occur due to non-communicable diseases (NCDs), three due to

communicable diseases and one due to injuries (The Global burden of disease: 2004 update: WHO report 2008). In 2008, 63 % deaths occurred due to NCDs such as cardiovascular disorders, cancer, diabetes and chronic respiratory diseases (Global status report on non-communicable diseases 2010: WHO report 2011). It has been reported that cardiovascular diseases account for most deaths occurring due to NCDs, that is, 17 million deaths annually, followed by cancer (7.6 million), respiratory diseases (4.2 million) and diabetes (1.3 million). It has been predicted that 52 million people will die annually due to NCDs by 2030 (Global status report on non-communicable diseases 2010: WHO report 2011). Apart from NCDs, infectious or communicable diseases form the other major category responsible for causing large number of human deaths worldwide (>25 %). Amongst infectious diseases, tuberculosis and malaria are most prevalent throughout the world with one-third of population affected by tuberculosis and an estimated one million deaths caused due to malaria, respectively (Hotez et al. 2004; Tuberculosis: WHO fact sheet 2010; Malaria: WHO Fact sheet 2008). Apart from these, there is a large group of “neglected tropical diseases”, including dengue, African trypanosomiasis, leprosy, schistosomiasis, and leishmaniasis, contributing to the global burden of infectious diseases. A recent WHO report stated that almost two-fifth of the world population is at the risk of contracting dengue and associated haemorrhagic fever while 249,007 new cases of leprosy were registered in 2008 mainly in Africa and Asia (WHO report 2010; Dengue: WHO Fact sheet 2009; Leprosy: WHO fact sheet 2010). The data indicates the need for developing better interventions, especially in the field of drug development process, for cure and prevention of such diseases.

As is evident from the data presented above, the development of better drug molecules has become a prime concern for human healthcare sector as the conventional drugs and prevention measures adopted till date are gradually turning ineffective in curing various diseases. On the other hand, several limitations are encountered by the pharmaceutical sector during the process of drug discovery and development such as lack of novel drug entities, inefficiency of novel drug molecules, lower bioavailability, and development of multi-drug resistant pathogens (Charles and Grayson 2004). At this juncture, synthetic biology provides a ray of hope for accelerating the process of novel drug development with the potential to overcome above mentioned bottlenecks. For instance, integration of the data generated through genomic and proteomic studies with artificially designed genetic circuitry will allow for better understanding of the gene expression profile of a pathogenic organism (bacteria or virus) enabling the researchers to identify novel drug targets and design drug molecules against them. Assembling, manipulating and

Fig. 1 Applications of synthetic biology in microbial biotechnology

regulating the metabolic pathways at molecular level via synthetic biology can provide an understanding of the onset and progression of various non-infectious diseases (such as cancer and diabetes) as well and develop remedies to cure them. Therefore, synthetic biology can act as a potential platform for developing cures against a wide range of diseases.

Microbial system remains the most suitable living system for developing drugs such as antibiotics and vaccines in vitro. The unicellular nature and a comparatively simpler genome of the micro-organisms allows easy handling and regulation of gene expression as compared to the higher organisms. Microbial systems such as bacteria and yeast have a short regeneration time, thereby, simultaneously generating a large number of cells and the protein/RNA/drug of interest in a lesser time span (Fussenger 2010). Culturing micro-organisms is not a labour intensive process; their minimal nutritional requirement makes the entire process cost-effective. Such advantages make micro-organisms an attractive host system for engineering complex metabolic pathways of higher eukaryotic organisms. Certain eukaryotic metabolites particularly those obtained from plants (secondary metabolites) have medicinally beneficial properties but their extraction and production is a tedious task, often requiring skill, labour and huge financial investment (Jones and Kinghorn 2005). Therefore, engineering metabolites of interest into microbial host machinery can enable larger extractions of bioactive therapeutic compounds.

As described, synthetic biology has the potential to offer novel drug targets and aid in development of new drug molecules for human therapeutics. As for any other field, commercialization of products for public utility is dependent on the progress and growth of research in synthetic biology. Patents under the intellectual property regime, collaborations between academia and industry and university spin-offs are vital for commercialization of synthetic biology based applications (Saukshmya and Chugh 2010; Kumar and Rai 2007). The issues pertaining to biosafety, biosecurity and the dangers that could arise by misuse of the microorganisms as bioweapons also need to be addressed in context of synthetic biology based applications.

Keeping this in view, a holistic approach has been adapted in the present study for evaluating the role of synthetic biology in drug development using microbial system. An attempt has been made to describe the various strategies that discuss both the present scenario as well as the future prospects for drug development via microbial approach using synthetic biology. The paper focuses on development of novel antibiotics and vaccines via synthetic biology. The role of synthetic biology in engineering of metabolic pathways for utilization of medicinally

significant compounds derived from nature and its impact on conservation of biodiversity has been also analyzed. Issues pertaining to intellectual property rights, accessibility of the products arising out of synthetic biology and the socio-ethical concerns related to synthetic biology have been dealt in brief in the later sections of the study.

Synthetic biology and antibiotics

The first antibiotic, penicillin, was discovered by Alexander Fleming in 1928 from the mold *Penicillium chrysogenum* (Ligon 2004). Discovery of penicillin led to a new era of medicine which enabled cure and prevention of diseases caused by pathogenic organisms. Gradually, the field progressed with the advent of other classes of antibiotics including β -lactams, macrolides, glycopeptides, aminoglycosides and quinolones (Clatworthy et al. 2007). It was speculated that even the most lethal of infections could be prevented or cured with these antibiotics; however, as new pathogens and multi-drug resistant pathogens evolved, various antibiotics that were speculated to be effective for a long term have been rendered inefficient for curing diseases. Therefore, novel drug discovery emerged as a primary aim of contemporary pharmaceutical sector.

It has been observed that the antibiotic drugs approved by FDA in the past few decades are mere incremental discoveries over the existing genre of antibiotic drug molecules indicating a lack of breakthrough innovations required to ease the burden of infectious diseases. The last decade witnessed the emergence of only two novel classes of antibiotics namely lipopeptide and oxazolidinone (Norrby et al. 2005). In 2006, a promising antibiotic molecule, platensimycin, was also discovered however, it could not reach clinical trials due to its poor pharmacokinetic properties (Pearson 2006; Martin and Demain 2011). Lipopeptide and oxazolidinones are active against gram positive bacteria such as vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus*. The two FDA approved drugs under these classes are Daptomycin and Linezolid respectively (Charles and Grayson 2004; Norrby et al. 2005). The emergence of only two novel classes of antibiotics in the otherwise “crowded” antimicrobial drug market is indicative of the declining pace of development of novel antimicrobial drugs.

The slowdown in the development of novel antimicrobial drug candidates can be attributed to several factors. The primary factor is the evolution of multi-drug resistance in pathogenic organisms. Such pathogens are resistant to a wide spectrum of antibiotics and hence cannot be eliminated easily from the host. The emergence of multi-drug resistant (MDR) *Mycobacterium tuberculosis* can be considered as a classic example in this context. The current

treatment for tuberculosis involves administration of a combination of four to five antibiotics such as Isoniazid, Rifampicin, and Ethambutanol (Chan and Iseman 2002). However, this treatment is not effective against MDR *M. tuberculosis* (Drug and multi drug resistant tuberculosis: WHO report 2011). Another factor that hinders the novel drug development process is the lack of enabling knowledge of the pathogenic organism as complete information about the genetic makeup, the regulatory genes and the metabolic pathways of various pathogens still remains unknown. A complete understanding of the pathogen genome and phenome would not only provide novel drug targets but will also aid in overcoming the multi-drug resistance barrier exhibited by the pathogen.

The development of MDR in pathogenic organism occurs due to differential expression of genes in response to the continuous exposure to an antibiotic. Synthetic biology can be applied to understand the gene expression profile of the pathogens and aid in achieving the objective of discovering novel drug targets and designing drug molecules against them. For instance, the sequence of the pathogenic genome along with the proteomic profile will help in developing customized devices such as oscillators, receivers and senders (to analyze the cell signalling pathway) and protein generators. It is envisaged that by assembling the customized genetic circuitry developed using the sequence information of the pathogen in a non-infectious micro-organism (e.g. *E. coli*) will help elucidate the infection cycle of the pathogen and aid in developing strategies to prevent the infection by the pathogen under investigation.

Apart from genome sequence data analysis, proteome profile and metabolic pathway studies of a pathogen for drug development, another strategy that has been explored is the development of adjuvants for pre-existing antibiotics (Lu and Collins 2009). An adjuvant is a molecule that enhances the efficiency of antibiotics by interfering with the survival pathways initiated by the pathogens in response to the antimicrobial compounds. Lu and Collins (2009) recently designed a system where a bacteriophage M13mp18 has been engineered with SOS response repressor, LexA3. Antibiotics such as quinolones, act by damaging the bacterial DNA. As a response to the DNA damage caused by the antibiotic, the SOS response pathway (DNA repair system) is activated. DNA repair increases the survival rate of bacteria as a result of which the antibiotic treatment becomes ineffective. Treating the phage infected bacteria with quinolones showed a decreased survival of bacteria (*E. coli*) under in vitro as well as in vivo conditions (Lu and Collins 2009). Similarly, adjuvants can be developed against other pathways that confer antibiotic-resistance to the pathogens. The development of such adjuvants will increase the potency and

efficacy of existing antibiotics leading to eradication of the pathogenic microorganisms. However, the possible reactions that may occur upon introduction of such products into the human body are highly unpredictable invoking the need for risk assessment, essential to understand the implications of synthetic biology.

Recently, efforts have been made at engineering the polyketide synthesis pathway in *E. coli* and *Streptomyces coelicolor* via synthetic biology (Gao et al. 2010; Medema et al. 2011). Polyketides is a class of antimicrobial compounds obtained from a wide range of organisms such as bacteria, fungi and plants (Gao et al. 2010). They are synthesized by a group of enzymes called polyketide synthases (PKS) which exist as a gene cluster that is known to be smaller than the gene cluster of other antibiotic compounds (Medema et al. 2011). The immense pharmaceutical value possessed by polyketides have made them an attractive target for understanding and engineering their biosynthetic pathway via synthetic biology. The information obtained via genome sequencing projects has provided a baseline for developing a synthetic assembly for PKS genes and transfecting them into an appropriate chassis (*E. coli* and *S. coelicolor*). Several devices and tools have been designed for expressing PKS genes specifically in *S. coelicolor* (Medema et al. 2011). Such systems can be considered as a model for manipulating the genetic mechanisms underlying the production of other antibiotic molecules.

Antimicrobial peptides and synthetic biology

Apart from the conventional classes of antibiotics that is, β -lactams, tetracyclines, macrolides, and quinolones, another major category of antimicrobial agents which has emerged over the years are antimicrobial peptides (AMPs). They are a class of peptides, usually <100 amino acids long, that interact with cell membrane components and exhibit antimicrobial activity. They can either be antibacterial, antifungal or antiprotozoal in nature. Antimicrobial peptides not only have a wide spectrum of action but they are also distributed widely across genera. AMPs can be found in bacteria, plants, mammals and humans (The antimicrobial peptide database 2011). They act as self-defense molecules against invading pathogens and have been employed for therapeutic purposes in vitro. However, no AMP has been approved by FDA for clinical applications yet (Gordon et al. 2005; Godballe et al. 2011).

Isolation of AMPs is challenging as they are produced and secreted in scarce amounts and hence the yields required for commercial scale may not be achieved. As an alternative, engineering of pathways leading to synthesis and secretion of AMPs in microorganisms such as *E. coli*

can be practiced via synthetic biology. Developing such a strategy would produce AMPs for commercial use that can be employed for human therapeutics, irrespective of their origin. The modulation of the genetic circuitry can be performed in such a way that a single bacterium is able to produce multiple AMPs conferring immunity against a broad spectrum of pathogens.

Synthetic biology for vaccine development

Vaccination is a process of immunizing an individual by stimulating the immune system of an individual to an antigen or to the non-virulent form of the microorganism responsible for causing disease (Kindt et al. 2000). There are several categories of vaccines that have been used to confer immunity: live, killed, attenuated, subunit and DNA vaccine (Kindt et al. 2000). Among these live, killed and attenuated are conventional types of vaccines while subunit and DNA vaccines are recent advancements in the field of vaccine development. Although newer types of vaccines have evolved, attenuated vaccines, till date, remain the most effective and popular amongst all. A major limitation associated with attenuated microorganisms is their tendency to revert to the virulent form that may result in causing disease though the rate of occurrence of such cases may be low (Robertson 1988).

Subunit vaccines are the second generation vaccines. Unlike the first generation vaccines, such as live or killed, subunit vaccines elicit the immune response by exposing the immune system to only a single antigen of the pathogen. Subunit vaccines circumvent the problem of reversal to virulent form since they cannot spread to unimmunized individuals by virtue of their non-replicative nature (Baxter 2007). Though safer than first generation vaccines, subunit vaccines have several drawbacks associated with them. First, since the antigen has to be produced through recombinant DNA technology, it is possible that the peptide/protein may not fold in the correct conformation and hence may not be able to elicit the required immune response. Second, the antigens being proteinaceous molecules are smaller in size than whole organism and hence are not efficiently recognised by the immune system alone. Therefore, to present the recombinant antigens to the immune system, conjugation to adjuvants is required which might be suboptimal for certain infectious diseases (Baxter 2007; Kindsmuller and Wagner 2011). More effective subunit vaccines can be developed by employing synthetic biology based in silico designing of novel immunogens with improved expression (Kindsmuller and Wagner 2011).

A third generation of vaccines, DNA vaccines, are in the process of research and development as they are known to overcome the stated drawbacks associated with subunit

vaccines. DNA vaccines comprise of naked plasmid DNA, encoding for an antigen. The protein arising out of the DNA sequence coding for the antigen is used to elicit the immune response in vivo. The advantages conferred by DNA vaccines are similar to subunit vaccines though they have their own limitations. The major limitation of DNA vaccines is their method of delivery to the target cells. Naked DNA cannot be directly injected into the blood stream and requires assistance of a vector system (Fioretti et al. 2010). As an initiative in this direction, via synthetic biology, a system has been devised wherein the bacterial transcription-and-translation network and a DNA encoding a model antigen has been reconstituted and encapsulated in liposomes. It has been reported that the system is able to express the antigen in vitro as well as in vivo and elicits humoral immune response in live mice (Amidi et al. 2011; Ruder et al. 2011). Another alternative approach for improvising the vaccine design based on synthetic biology is synthetic attenuated virus engineering as described below.

Synthetic Attenuated Virus Engineering or SAVE is a technique in which viruses are re-encoded such that the wild-type amino acid sequence for the antigen is retained with simultaneous rearrangement of the codon pairs to obtain attenuated form of the virus (Coleman et al. 2008). Virus engineering involves utilization of the tools and concepts applied in synthetic biology. The virus thus obtained can serve as a vaccine for preventing viral infection due to exposure to its wild-type form. Such vaccines have been already developed and tested in vitro against influenza and polio, and await commercialization (Coleman et al. 2008; Mueller et al. 2010).

Another very interesting application of synthetic biology in relation to development of antimicrobial drugs and vaccines is the resurrection of genomes of pathogenic organisms that have been eradicated. For example, reconstruction of the genome of Spanish Influenza virus has been carried out via synthetic biology approach (Wimmer et al. 2009). It is noteworthy that reconstruction of genomes provides an insight into the virulence mechanism of the extinct pathogens and a comparative analysis of their genetic makeup vis-a-vis their contemporary counterparts can also be studied. Such investigations can aid in developing vaccines or drugs for combating any future disease outbreaks that may be caused due to their variants. Preservation of the small pox virus genome has been the first step towards developing a library of such pathogenic agents (CDC 2002). Though as a foresight, it may have various beneficial applications; such steps still pose a risk for the security of a nation, making it pertinent to address the security, social and ethical concerns of novel synthetic biology applications. Such issues have been discussed in the later sections of the present study.

Based on the knowledge-base developed from viral vaccine research via synthetic biology, it is possible to develop vaccines against bacterial infections also. A probable strategy for modulating the genetic makeup of a bacterium could be modification of codon pairs via site-directed mutagenesis (Baronio et al. 2010). Such a modification can act as a cue for developing vaccines against bacterial pathogens by altering the pathogen at the molecular level.

Metabolic engineering with synthetic biology

Unlike SAVE, which modulates the codon usage for building proteins in microbes, metabolic engineering involves modification of the existing metabolic pathways or incorporation of a new metabolic pathway in an organism for various applications in pharmaceutical and cosmeceutical industry (Yang et al. 1998). Metabolic engineering of medicinally important plant species is an area of active research in the pharmaceutical sector for novel drug development. Plants have been known to produce secondary metabolites such as alkaloids, and terpenoids that possess various medicinal properties. Although commercially valuable, the plant secondary metabolites are produced in minute quantities. Therefore, attempts are being made by the researchers to enhance the yield of these naturally occurring therapeutic compounds.

One of the first methods employed to achieve this goal was vegetative propagation employing explants such as leaf, roots and apical buds, under controlled conditions, referred to as micropropagation. The noteworthy advantage associated with micropropagation is that from a single parent plant, geometric numbers of “clones” of the same plant could be obtained (Micropropagation Technology 2011). However, there are various limitations associated with micropropagation. First, it is a labour-intensive process and requires skilled personnel. Second, there are several plant species which are not amenable to micropropagation and hence rendered recalcitrant to plant tissue culture techniques. Third, for plants such as *Catharanthus roseus*, a well known source of anticancerous agents (vincristine and vinblastine), the ultimate objective of obtaining high yields of the medicinally active compound is not achieved since the active compound is either not produced or produced in even lesser amounts as compared to its wild-type form via micropropagation (Rijhwani and Shanks 1998).

The second strategy that has been employed in vivo to enhance secondary metabolite production is metabolic engineering. Attempts have been made to regulate the metabolic pathways by manipulating the expression of genes involved in the biosynthesis of medicinally active

compounds in medicinally important plants. It involves generation of transgenic plants transformed with multiple genes expressing the desired proteins (Yun et al. 1992). This approach is industrially non-favourable because it is experimentally cumbersome and cost-intensive. Therefore, a third strategy, based on synthetic biology aims at engineering the complete pathway of metabolite production into microbial systems (Martin et al. 2003). As mentioned previously, microbial systems offer the advantage of easy handling, better quality control, regulation of gene expression profile and obtaining high number of cells, leading to high yield of the product of interest, in a short span of time. The proteins or metabolites can be purified easily from microorganisms as compared to the other experimental systems.

Engineering of metabolite pathways in microbial systems can be performed by sequentially incorporating the genes, as they function in the natural system, into a cassette of plasmid vectors. The promoter elements have to be regulated such that the final product is obtained in higher yields. For example, artemisinin is a compound with anti-malarial properties that can be extracted from *Artemisia annua*. It is difficult to isolate artemisinin in significant quantities from plant extracts. Its biosynthetic pathway has been engineered by Jay Keasling and co-workers in *E. coli*. The precursor of artemisinin, artemisinic acid, is synthesized in *E. coli* and extracted followed by its conversion to artemisinin chemically (Martin et al. 2003). A similar strategy can be adopted for increasing the yield of several other naturally derived medicinally active compounds. For example, vincristine and vinblastine, the anti-cancerous compounds from *C. roseus* can be produced in large quantities at industrial level by using a similar strategy. Vindoline, a toxoid, is the precursor of vincristine and vinblastine. It is formed as a product of several reactions combining the amino acid tryptophan and a metabolite of sterol synthesis, secologanin (Sayed and Verpoorte 2007; Ziegler and Facchini 2008). The enzymes involved in the synthesis of vindoline can be cloned and arranged as a cassette such that there is sequential and appropriate expression of all enzymes, followed by expression in a suitable bacterial system (Fig. 2).

Exploiting traditional knowledge: role of synthetic biology

As described in the earlier section, like artemisinin and vincristine, other naturally occurring medicinally important compounds can also be exploited for drug discovery and development against several diseases. A vast reservoir of traditional medicinal knowledge rests with the natives of biodiversity rich nations such as India. There are approximately 400,000 species of plants of which 7,500 are

Fig. 2 Metabolic pathway for synthesis of vincristine and vinblastine in *Catharanthus roseus* (The enzymes involved have been mentioned sequentially)

known to be medicinally important (Botanic Gardens Conservation International; Bhandari et al. 2008). For example, plants such as turmeric and neem (*Azadirachta indica*) produce curcumin and nimbidin respectively, which have antiseptic, antipyretic and anti-inflammatory properties (Biswas et al. 2002). Similarly there are several plant derived compounds which are also known to treat diseases such as cancer (taxol, vincristine and vinblastine), malaria (quinine, artemisinin) and leprosy (alcoholic extract of *Plumbago zeylanica*) (Martin et al. 2003; Guo et al. 2006; Aslam et al. 2010; Beg and Ahmad 2000). Plant derived compounds can serve as important lead molecules for the treatment of communicable as well as non-communicable diseases.

However, direct isolation and extraction of medicinally active compounds from medicinal plants in significant amounts requires harvesting of the native plants at a large scale. Such activity potentially disturbs the ecological balance of a biome either by mass harvesting or by promoting monocultivism. For example, taxol, an anticancerous agent derived from the yew tree (*Taxus brevifolia*) has been approved by FDA for treatment of tumors (Guo et al. 2006). However, obtaining one gram of taxol requires sacrifice of three yew trees (Small and Catling 1999). Since yew tree is an extremely slow growing conifer, it may take hundreds of years to replenish such vegetation of yew trees. Also, it will have a negative impact on the ecological habitat and may lead to extinction of some rare species of plants, insects and animals which survive on yew trees (Ferr 2008). Hence, production of metabolites in alternative living system such as microbes using synthetic biology

based approach will be an effective measure for sustainable utilization of resources and technology in the pharmaceutical sector.

As can be observed from the earlier sections, synthetic biology offers plethora of opportunities for improving human lives through effective theragnostics. Figure 3 illustrates potential application of synthetic biology using microbial systems in different areas of healthcare such as production of therapeutics, vaccine development and drug delivery. To reap maximum benefits and sustainable growth of synthetic biology, close scrutiny of its associated implications is recommended. The sections below examine the socio-ethical and legal issues/challenges associated with synthetic biology.

Synthetic biology: safety, security and accessibility

As described earlier, synthetic biology is a multidisciplinary area with a wide range of applications including clean technologies and human therapeutics. Manipulation of microorganisms via synthetic biology for the new use requires an assessment of the limitations, challenges and the anticipated risks against the human society and environment. Two major risk loci pertaining to synthetic biology based products are biosafety and biosecurity. While biosafety focuses on both ecology and human health impact of the manipulated microorganisms, biosecurity pertains to malicious use of such organisms. Patent law regime also takes these two issues into consideration, thereby, prohibiting patenting of inventions that may harm

Fig. 3 Biomedical applications of synthetic biology for human healthcare

human, animal or plant health or the environment. A critical analysis of these issues at this point of time is important, when the research is progressing and the products shall be launched in the market in the near future. The present section deals with biosafety, biosecurity and intellectual property issues specific to the use of synthetic biology in therapeutics, using microbial systems.

Biosafety

Emerging technologies carry an inherent risk in terms of biosafety at all stages of its development. Therefore, it is essential to assess the magnitude and type of risks that may be associated with evolving synthetic biology based research, particularly its application in the field of therapeutics. Due to the ease of propagation of microorganisms and lack of stringent safety measures for virulent strain containment, in case of organisms engineered using synthetic biology techniques, pose an inherent risk of spread to non-targets increasing the probability of development of exotic species (Norton 2010; The New Biomasters: ETC. report 2010). Transmission of virulent strain through needle pricks or air and inability of the technology to control the spread of the microorganism is the main cause of concern for biosafety as well as biosecurity (Gutmann et al. 2010). Presently, it is difficult to effectively ascertain risk and impact assessment of the therapeutics produced by use of synthetic biology in microbial systems as research in this field is still in an early phase. The present study discusses the current status and efforts made by various nations for governance of synthetic biology to mitigate its potential risks. To understand the implications of synthetic biology, mainly, Europe and USA have taken various initiatives as described in the following paragraphs.

Europe has laid down significant emphasis on biosafety aspects as well as public engagement with the subject. Three policy development activities have been ongoing in

Europe since 2007 these are -Towards a European Strategy for Synthetic Biology (TESSY), SYNBIOSAFE and EMERGENCE (UK. The parliamentary office of science and technology. Synthetic Biology Postnote 2008). SYNBIOSAFE among these activities is responsible for examining the ethical, safety and security issues of research. Debates regarding the societal aspects of synthetic biology were stimulated by SYNBIOSAFE to understand ethical, safety and security implications (Schmidt et al. 2008) and it was concluded that new methods for risk assessment of synthetic biology applications are needed along with safer biosystems to reduce the risks. The other two are mainly associated with standardising the research activities related to synthetic biology.

Both the reports of European Academics Science Advisory Council—Realising Potential in Synthetic Biology: Scientific opportunities and good governance December 2010 (Meulen 2010) and Review report commissioned by Biotechnology and Biological Sciences Research Council (BBSRC) 2008 (Balmer and Martin 2008) emphasises on the following two considerations:

1. The scientists and researchers should clearly communicate the implications of the research at various stages.
2. The innovators should adhere to a code of conduct enabling self regulation, for effective governance of the synthetic biology.

Biosafety implications should be observed and recorded by the scientists during the course of research. Clear and timely communication regarding the implications would help in controlling the risks aptly. Development of the code of conduct and adherence to it, would serve as an option for self regulation of research activities by the scientists and researchers that would further enable effective governance of synthetic biology. Further, self regulation needs to be complemented with a framework regulating both research and commercialisation of the results.

In USA, the Presidential Commission for the study of Bioethical Issues (Gutmann et al. 2010) in December 2010, upon the request of the President, examined the possible implications of synthetic biology. According to the Commission, it is important to consider carefully the potential risks of synthetic biology applications to humans, other species, nature and environment. But at the same time, the Commission recommends that as the field is at a nascent stage of development, efforts towards encouraging innovation should be made. In order to promote synthetic biology, review of existing policies and practices should be the next significant step. Study by the Commission suggests that the collaborative efforts of academicians, government and private sector scientists should be directed towards evaluation of the field for risk containment. As

described above, one of the major concerns, lack of control over the spread of engineered microorganisms, has been addressed by the Commission. As a solution, to ensure limited life span of the organisms, the use of such gene sequences that would trigger self destruction thereby inhibiting proliferation and spread of these organisms could be developed. Various strategies need to be envisaged to screen and combat the potential risks that arise in due course of development of synthetic biology. To minimise such risks a framework to regulate synthetic biology based research and to assess the biosafety of the product pre/post release is essential.

A three year project from April 2009 to March 2012 has been funded by the National Natural Science Foundation of China and the Austrian Science Fund to analyse the Chinese and Austrian biosafety and risk assessment needs of synthetic biology at the national level. Based on the findings of the review the existing regulatory framework would be improved in both the countries (Investigating the biosafety and risk assessment needs of synthetic biology in Austria and China 2011). India, an emerging knowledge based bioeconomy and many other developing countries also eventually need to develop legislation as well as policies to regulate this emerging area of synthetic biology that has various potential commercial prospects.

Biosecurity

It is noteworthy that synthetic biology possesses the potential of improving quality of human lives, at the same time, it is capable of producing microorganisms that may be harmful to the humans as well as other organisms, raising the question of biosecurity. Ensuring national security and well being is the main aim of government of any nation. Therefore, it becomes crucial for the policy makers to consider the biosecurity aspect of any new technology. The National Science Advisory Board for Biosecurity, USA (NSBB 2011) defines “Biosecurity” as the protection, control of and accountability for high consequences biological agents, toxins and critical relevant biological materials and information, to prevent unauthorised possession, loss, theft, misuse, diversion or intentional release.

As discussed in earlier section, synthetic biology offers the opportunity of engineering the microorganisms for developing novel drugs, vaccines and may further involve resurrecting the extinct pathogens such as Spanish influenza virus. Dual use nature of synthetic biology raises a concern of misuse. Regular assessment, monitoring and surveillance of synthetic biology applications can aid in preventing misuse of the technology towards antisocial activities such as bioterrorism. Strategies and policy frameworks need to be in place to effectively control the

misuse of synthetic biology. One such framework is ‘The Screening Framework Guidance for Providers of Synthetic Double Stranded DNA’ (2010) developed by US government in 2010. The framework recommends baseline standards for the gene and genome synthesis industry as well as other providers of synthetic double stranded DNA products. Nucleic acid sequences can be used for developing novel virulent microorganisms. Such particular sequences can be misused and hence termed as ‘*sequences of concern*’. In order to identify sequences of concern, of any length, that may be specific to the listed select agents or toxins on the National Select Agent Registry, screening is necessary. The framework recommends three levels of screening, customer level, sequence level and finally follow up screening, mainly to know the requestor of the order and if the sequence requested in part or on whole is a ‘*sequence of concern*’. The Guidance also recommends proper records retention, protocols as well as the sequence screening software that must be selected by synthetic double stranded DNA providers. US Government recommends selection by the providers of a sequence screening software tool that utilises a local sequence alignment technique for the best match. A general screening method to be followed by the service provider for automatic identification of potentially dangerous sequences has been outlined in the document. However exact implementation of the method is difficult due to inadequate instructions provided in the Federal Guidance Framework. To overcome the limitations of the Guidelines, Virginia Bioinformatics Institute has developed software in open source format called GenoTHREAT. The software is based on the best match screening protocol recommended by the Federal Government to minimise the risk (Adam et al. 2011). As suggested by the SYNBIOSAFE, researchers can contribute towards regulation of synthetic biology by developing safe systems and ensuring judicious use of the synthetic biology applications for the benefit of mankind.

On similar lines of the screening framework, two other competing standards have been issued by the International Association Synthetic Biology (IASB) (ISAB 2011) and the International Gene Synthesis Consortium (IGSC) (IGSC 2011). The codes provide guidelines for the development and implementation of sequence screening tools as well as mechanisms for reporting and resolving concerns about the orders of potentially dangerous sequences. The codes also emphasize compliance with the national regulations on potential dangerous nucleic acid sequences, by the entities and companies involved in commercial or non-commercial synthesis of DNA sequences. However, all these frameworks need to be developed further to ensure uniformity.

In the knowledge-based economy, intellectual assets such as patents serve as an impetus towards technology

development and economic growth. It is a debatable issue whether patents in the field of synthetic biology applied for production of therapeutics through microbial system, would open or restrict channels for its commercialisation and growth. The following section explores the scope of protection for the synthetic biology manipulated microorganisms under intellectual property regime and open accessibility of the synthetic biology.

Intellectual property regime versus accessibility of synthetic biology

Simple, low-cost production of biopharmaceuticals, novel drug delivery systems and effective therapies for chronic incurable illnesses are some of the focal research areas of synthetic biology in the healthcare domain. As a result there is significant market potential for such products in the pharmaceutical sector. Partnerships and collaborations between industry and academia would contribute in realising this potential. As mentioned previously intellectual property regime in general and patents in particular will have a significant role in commercializing synthetic biology. The present section intends to explore the scope and limitations of the patent regime with respect to innovations resulting from synthetic biology based applications in the microbial system for therapeutics.

The number of patent applications filed in the field of synthetic biology is increasing constantly (Saukshmya and Chugh 2010). Diverse subject matters have been protected under patent regime ranging from methods of developing synthetic DNA strands, compositions, genes or parts of genes to the methods of metabolic engineering. Most of the patent applications are based on broad claims (Saukshmya and Chugh 2010). The scope of protection of the inventions related to production of therapeutics by microbial systems using synthetic biology may include product as well as the process. The product can be new antimicrobials or other compounds produced by the manipulated microbe, or the modulated organism or the engineered microorganism acting as a therapeutic itself. Establishing the novelty and non-obviousness of the already known metabolite would be difficult, thereby omitting the scope of protection in all the jurisdictions. However the manipulated microorganism can be protected. According to TRIPS agreement genetically modified microorganisms are patentable. The landmark US Supreme Court judgement in *Diamond v. Chakrabarty* changed the future of patenting of artificially created microorganisms (*Diamond v Chakrabarty* 1980). In brief, a patent application related to a genetically engineered bacterium capable of breaking down crude oil, a property which is possessed by no naturally occurring bacteria was filed by Prof. Anand Chakrabarty. Patent Office Board of

Appeals affirmed patent application claim rejection by the patent examiner on the ground that living things are not patentable subject matter. Interestingly, this affirmation was reversed by the US Supreme Court which stated that a new bacterium with characteristics markedly different from the one found in the nature was created; thereby the invention in question involves human intervention. The decision of the Supreme Court paved way for patenting genetically modified microorganisms and extends the scope of protection in USA to the microorganisms manipulated using synthetic biology techniques. Even other countries such as Europe and India extend the same scope of protection to synthetic biology based products specially microorganisms as USA. In Europe, considering that synthetic biology inventions deal with biological material, same patentability criteria are applied as that of biotechnology inventions. As there are no provisions in the European Patent Convention, governing the non-biological systems, a need to differentiate the rules for synthetic biology is important (Berthold 2010). All signatories of World Trade Organisation (WTO) adhere to the Trade Related Intellectual Property Rights (TRIPS) agreement. They are under obligation to extend protection for microorganisms, non-biological, and microbiological processes under the patent law. Article 27(3)(b) of TRIPS Agreement allows member states to deny patents for “plants and animals, other than microorganisms, and essentially biological processes for the production of plants or animals other than non-biological and microbiological processes.” India being a signatory member of WTO amended the Indian patent legislation to be TRIPS compliant. In India manipulated microorganism and method of manipulation are patentable. As per the section “[Antimicrobial peptides and synthetic biology](#)” (j) of the Indian Patent (Amendment) Act, 2005, microorganisms do not fall under the non-patentable inventions:

Plants and animals in whole or any part thereof other than micro-organisms but including seeds, varieties and species and essentially biological processes for production or propagation of plants and animals.

As described in the earlier section, naturally occurring medicinally important compounds identified on basis of traditional knowledge are exploited for drug discovery and development against several diseases. Instead of isolating the compounds from the plants, the microorganisms can be engineered with pathways to produce the therapeutic compounds (secondary metabolites in this case). Patent protection of such traditional knowledge based engineered microorganisms would vary in each jurisdiction. In India inventions based on traditional knowledge are not patentable under the Indian Patent Act, 1970, while in USA and Europe such inventions are

patentable. Lack of novelty and inventive step in the invention may render them non-patentable as most of the methods involved in developing the organisms for therapeutic production are standard procedures. The nucleic acid sequences are the major elements of the invention, extension of protection to these would be subject to fulfilment of the patentability criteria.

On the other hand, extensive patenting of processes and techniques may prove detrimental to the growth of synthetic biology. It may lead to complexity due to patent thickening which may inhibit the freedom to research and commercialisation of synthetic biology based therapeutics. Policy makers and researchers alike are concerned with grant of broad claims that would further contribute to the restrictive environment for research due to involvement of interdisciplinary examination of patents (Saukshmya and Chugh 2010). European Academics Science Advisory Council (Meulen 2010) has already urged the European patent office not to grant broad claims and suggests the researchers to develop mechanisms that can promote collaborations, an environment to ensure consistent innovations in synthetic biology.

At the early stages of an evolving field such as synthetic biology, most of the scientists advocate the open access approach to promote exchange of ideas and collaborations.

The debate on intellectual property protection versus an open access mechanism is gaining momentum and has been analysed in the present study with respect to synthetic biology for therapeutics using microbial systems. It has been critically debated in various forums that an open access collaborative mode of research would be a better option at early stage of the technology as it will help it grow further. For designing therapeutic compound producing microorganisms, different tools and databases are required where open access can play a significant role in building up a strong synthetic biology based application platform. Open access enables researchers to cope with accelerating innovation cycles and multiple technologies necessitating regulation of such system. Different mechanisms to regulate the open access such as open source licenses, shrink wrap, click wrap licenses have been proposed (Saukshmya and Chugh 2010, 2011; Rai and Boyle 2007). Recognition to an innovator can be secured by imposing an obligation on the user to acknowledge the inventor, but how far this can be achieved remains uncertain. As an alternative, *sui generis* system that would protect the interest of the innovators for a shorter duration and promote further innovation in the field, will aid in regulation of open access. The system can be based on the utility model concept wherein the lower level of novelty and inventive step is acceptable. Implementation of such a system requires critical analysis of every aspect of synthetic biology including its various applications.

Future perspectives

Whether synthetic biology should be considered as an independent discipline or a part of biotechnology is still a topic of debate in the scientific community. As per the definition provided by NCBI, biotechnology is the “body of knowledge related to the use of organisms, cells or cell-derived constituents for the purpose of developing products which are technically, scientifically and clinically useful” (Biotechnology 2011). Synthetic biology serves an important arm in realising the essence of biotechnology and hence can be considered as “advanced biotechnology” or extreme engineering. In the current research scenario, synthetic biology extends “the spirit of genetic engineering” by laying emphasis on gene clusters and products derived from them rather than the conventional genetic engineering which dealt with engineering of single genes and products thereof. However, synthetic biology will be able to savour its true essence if the researchers are able to create an organism from “scratch”.

Although synthetic biology is still in its nascent stage of development, it has immense potential to provide a platform for realising the goal of sustainable use of resources, improving human health and environment. Its multidisciplinary facet offers numerous opportunities and strategies in the arena of drug discovery and development. Synthetic biology based human therapeutics hold immense commercial value for the pharmaceutical sector as indicated by the rapidly increasing number of patents in this field.

Bacterial systems can be re-engineered and made to act as drug delivery vehicles apart from acting as storehouse of drug molecules. Bacterial circuitry can be modulated in such a way that the bacteria has surface receptors which would recognize specific target tissues or cells and will have the capability of synthesizing the drug molecule upon recognition. The bacterial cells can be given as a part of a pro-biotic formulation such that when it enters the human system, it circulates through the blood stream, reaches and recognizes the infected or diseased cells, followed by release of the drug at the target site. A pathway can also be engineered in the bacterial system such that it destroys itself after releasing the drug at the site of infection (Science Daily 2009).

Another potential area where synthetic biology using microbes can be applied is the study of protein conformation and disorders arising due to misfolding of proteins in a cell. The microbial cells can be made to synthesize proteins with differential codon usage followed by study of conformational changes occurring in the proteins (Moroder and Budisa 2010). Such experiments can provide an understanding of the mechanism underlying protein aggregation related disorders such as Alzheimer’s disease and Parkinson’s disease.

Despite its promising applications especially in production of drugs and therapeutics, synthetic biology raises concerns about risks to human health and the environment. Dual use concerns arise when research undertaken for legitimate scientific purpose has the possibility to be misused. Such misuse can pose threat to public health and national security. The channels of communication should be kept open even at the early stages, among the researchers, public and government to mitigate such emerging threats of synthetic biology.

Microbial systems being simpler organisms, as compared to eukaryotes, serve as model systems for developing tools for synthetic biology. Currently, the plasmid vectors, gene cassettes, promoter elements and regulatory subunits are under construction for use in microbial systems. The tools for engineering the genetic circuitry are available at BioBricks Foundation and registered as “Registry of Standard Parts” (Biobricks foundation 2011). Such initiatives encourage participation of scientist at all levels in order to provide a global platform for sharing the resources, tools and products available with scientists worldwide and promote further research in synthetic biology. A platform for young scientists entering the field of synthetic biology has been provided as Internationally Genetically Engineered Machine Competition (iGEM) that promotes sharing and reduction of novel ideas to products (iGEM 2011).

Such platforms promote free exchange of information and tools thereby enhancing breakthroughs as well as incremental developments in the field. Technical difficulties or costly research and development can be minimised by promoting alternative strategies. Lack of openness and communication can impede advancement in any field and synthetic biology is no different in this regard. Open access approach can prove a successful strategy along with intellectual property regime for the growth of synthetic biology. Patenting in the early research phase, public private collaborations, joint ventures, spin outs from the universities and complementary technology in-sourcing during R&D phases are some of the currently practiced strategies that have added futuristic value to synthetic biology. As discussed in earlier section and by Saukshmya and Chugh (2010) different subject matter ranging from methods of developing synthetic DNA strands, compositions, genes or parts of genes to the methods of metabolic engineering can be patented. Spin outs have the advantage of close association with the university simultaneously encouraging entrepreneurship in synthetic biology. A leading example of spin out in the field of synthetic biology for the production of therapeutics is the University of California’s Amyris Biotechnologies. A public private alliance such as the Synthetic Genomics Vaccines Company formed by Synthetic Genomics Inc. and the Craig Venter Institute with Novartis for production of flu

vaccines is another way of gaining commercial success for synthetic biology.

In conclusion, the above mentioned strategies have the potential to promote commercial success of synthetic biology along with a stringent patent enforcement regime or sui generis system that is a combination of open access and minimum duration monopoly. Growth of synthetic biology in the field of novel therapeutics via microbial approach is likely to have beneficial influence of the described strategies.

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