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Rising influence of synthetic biology in regenerative medicine

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Angharad Evans, Elizabeth Ratcliffe ✉

Centre for Biological Engineering, Department of Chemical Engineering, Loughborough University, Loughborough, Leicestershire, UK
✉ *E-mail: E.Ratcliffe@lboro.ac.uk*

Abstract: Synthetic biology is an emerging area of research that combines the investigative nature of biology with the constructive nature of engineering. Despite the field being in its infancy, it has already aided the development of a myriad of industrially and pharmaceutically useful compounds, devices and therapies and is now being applied within the field of regenerative medicine. By combining synthetic biology with regenerative medicine, the engineering of cells and organisms offers potential avenues for applications in tissue engineering, bioprocessing, biomaterial and scaffold development, stem cell therapies and even gene therapies. This review aims to discuss how synthetic biology has been applied within these distinct areas of regenerative medicine, the challenges it faces and any future possibilities this exciting new field may hold.

1 Introduction

Since its inception, the field of synthetic biology has endeavoured to make the engineering of biology more reliable, efficient and predictable with the aim of expanding the range of possible biological functions for research and therapeutic applications [1]. It seeks to solve existing problems by constructing new and improved models as opposed to relying on analysis and observation alone [2], thereby distinguishing itself from traditional biological approaches. It is because of this that synthetic biology has greatly influenced the advancement of the field of regenerative medicine. Some of the key milestones can be seen in Fig. 1 [3–34].

Regenerative medicine is an interdisciplinary area of research and clinical applications that facilitates the repair, replacement and regeneration of cells, tissues or organs to restore impaired function resulting from trauma, ageing, congenital defects or disease [35]. Unlike the transplantation and replacement therapies that were previously used, regenerative medicine uses a combination of several existing and newly emerging converging technological concepts. Traditional methods in regenerative medicine have relied on utilising the natural, evolved behaviour of human cells, thereby supporting and stimulating the body's own self-healing capability. When combined with synthetic biology, the potential to improve our ability to help those in clinical need greatly increases [36] and novel solutions are presented to help combat several of the challenges currently faced within the field [37].

Such challenges include (i) the need for intercellular communication and spatiotemporal coordination within three-dimensional tissue-engineered constructs [38, 39]; (ii) the limited proliferative capability and plasticity of adult stem cells and the need to achieve adequate cell numbers for therapeutic applications through extensive *in vitro* expansion [40, 41]; (iii) the nonspecific pleiotropic effects of cytokines, extracellular matrix (ECM) molecules and growth factors on lineage fate determination and cellular differentiation [42]; and finally (iv) the safety issues associated with the genetic modification of human stem cells and the use of viral vectors and recombinant DNA [43]. These clinical challenges have also been critically examined in detail elsewhere [37].

With these challenges in mind, synthetic biology can be applied to several areas within regenerative medicine, including cell, tissue and organ transplantation, tissue engineering, bioprocessing and control, biomaterials and scaffolds, stem cell therapies, and gene therapies. This review aims to examine how synthetic biology has affected these various aspects of regenerative medicine, how it could be applied or improved in the future, and also some of the challenges faced within this promising new field.

2 Synthetic biology and tissue engineering

Given the unique ability of stem cells to provide trophic support and replace dying cells [44], their manipulation is one of the main methods used when approaching challenges within regenerative medicine. Examples of this include the addition of stem cells to damaged or diseased tissue to promote healthy tissue growth, *ex corpora* manufacture of new organs from stem cells followed by transplantation, the addition of mesenchymal stem cells (MSCs) to secrete cytokines, trophic factors and growth factors to signal for regeneration in endogenous cells; and finally repairing tissues following pharmacological intervention by stimulating the differentiation of stem cells or the division of healthy tissue surrounding a damaged site [45].

Due to this, tissue engineering is one of the main areas within regenerative medicine that has greatly improved since the implementation of synthetic biology, as shown in Fig. 2. Where the cross-over of scientific and technological advances, methods and techniques such as gene circuits for easier and enhanced manipulation of cells and cell signalling has and continues to impact upon the field of regenerative medicine and tissue engineering [6, 23, 25, 27–29]. Within this last decade, however, one of the most notable breakthroughs in tissue engineering was achieved by Baiguera *et al.* (2010). Through using an optimised detergent-enzymatic method (25 cycles) to decellularise cadaver tracheal tissue, they were successful in creating a bioengineered trachea that was structurally and mechanically similar to native trachea, and contained angiogenic factors that exerted chemo-active and pro-angiogenic properties. This structure was obtained within three weeks as opposed to the typical three months, thereby allowing the development of clinically functioning, fully *in vivo* tissue engineered airway replacements that can be obtained within a clinically useful time [23, 46]. In saying this, research is still underway to develop improved human tissue structures through the use of novel state-of-the art technologies and methods, for instance three-dimensional (3D) printing, 3D-biofabrication approaches (a combination of

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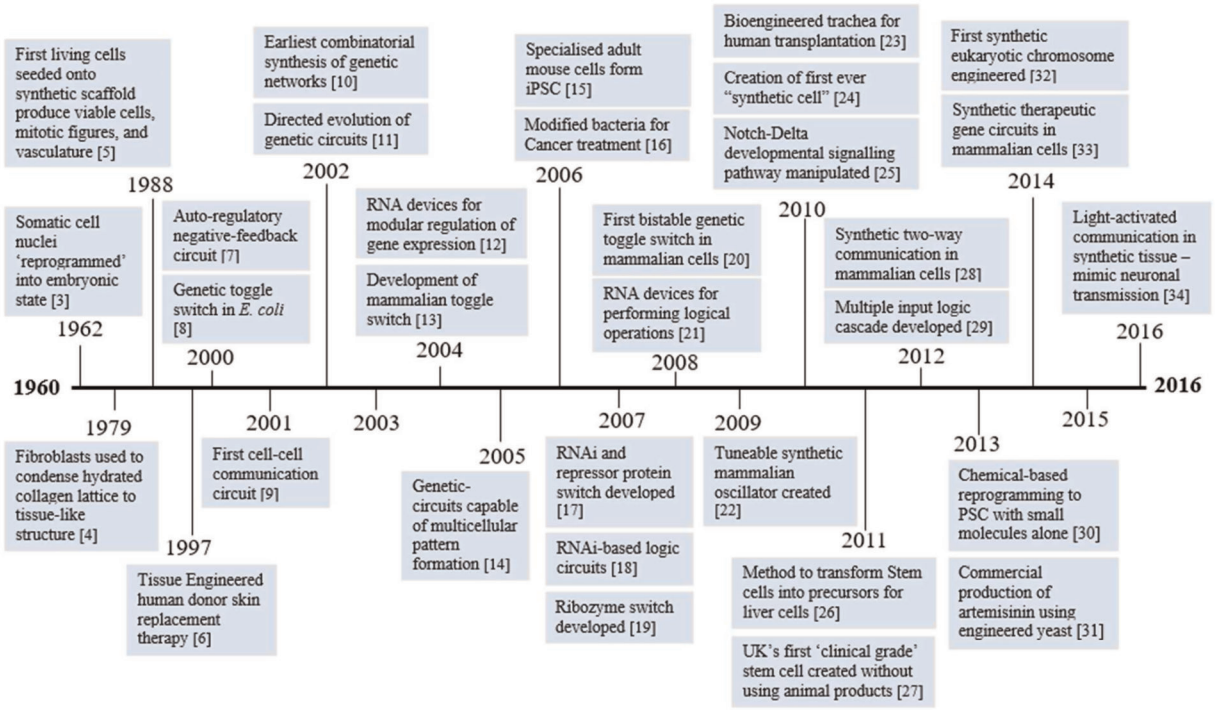


Fig. 1 *Brief timeline of some of the key milestones in synthetic biology with regards to regenerative medicine and the emerging healthcare industry*

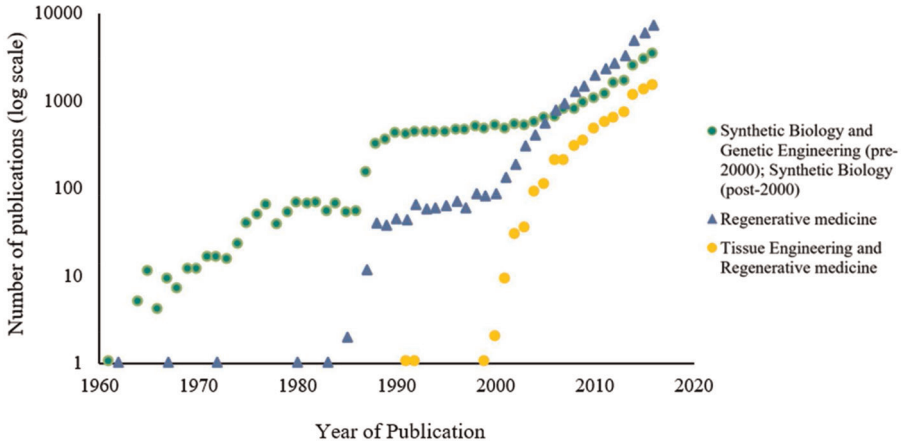


Fig. 2 *Rises in synthetic biology and genetic engineering (pre-2000) and synthetic biology (post-2000) (green), regenerative medicine (blue) and tissue engineering in regenerative medicine (yellow) as shown by the number of publications released. The term synthetic biology as used today under current definition was created around early 2000 therefore this term was used for searching post-2000 literature, but pre-2000, the terms genetic engineering and synthetic biology were used to be more representative of the emerging field. Graph was obtained using PubMed online search database, a search was conducted using YYY, XXX, where YYY represents the search terms synthetic biology and genetic engineering (pre-2000), synthetic biology (post-2000), regenerative medicine, tissue engineering regenerative medicine. XXX represents the range of publication release dates, from 1960 to 2016*

3D-bioplotting and thermally-assisted forming) [47, 48], and the generation of orgainoids by bioreactor-assisted self-assembly methods [49].

3 Materials and scaffolds

Another aspect of synthetic biology that has greatly aided the field of tissue engineering is the development of synthetic biomaterials and scaffolds.

Biomaterials play a pivotal role in modern day regenerative medicine and tissue engineering approaches. The natural or synthetic polymers used to produce the biomaterials and scaffolds act as designable and biochemical milieus [50] that elicit specific cellular functions [51] and facilitate the attachment, guidance, and differentiation of cells *in vivo* [52–54]. Being able to manipulate both the physical and chemical properties of the scaffolds allows the customisation and innovation not offered by naturally occurring materials. The guidance provided promotes the restoration of structure and function of damaged or dysfunctional tissues in both cell-based and acellular therapies [50], the development of a biomineralised scaffold for the repair of bone and other hard tissues is a prime example of this [55].

By implementing synthetic biology into the design and creation of biomaterials and scaffolds, it has led to the development of an array of biologically compatible synthetic materials which, when compared to naturally occurring materials, significantly reduce the risk of carrying biological pathogens or contaminants into the host [56]. Such innovative biomaterials, developed in the last decade, include novel synthetic peptide-based biomaterial scaffolds [56],

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interactive biohybrid materials [57–59] and the development of semi-synthetic ECMs from covalently cross-linked biodegradable hydrogels [60]. This semi-synthetic ECM is designed to allow the inclusion of the specific biological cues required to simulate the complexity of the ECM of a given tissue. Prestwich [60] suggested that the development of such a scaffold offers a manufacturable, flexible, highly reproducible, affordable, and Food and Drug Administration (FDA)-approvable vehicle for cell expansion and differentiation in a 3D environment, in theory making it ideal for clinical applications. Although these semi-synthetic ECMs are currently still in the research and developing stages of the development pipeline, some are currently being used as research tools for 3D culture of stem cells, primary human cells and orthotropic tumour xenografts. They are also being marketed as products for veterinary wound care and bone repair [61]. In addition to this, in 2015 the first synthetic in situ cross-linkable hyaluronic acid (HA) hydrogel, similar to the one used to create the semi-synthetic ECM, entered clinical trials for the treatment of HIV-induced lipoatrophy [62], thereby showing promise for the use of synthetic or semi-synthetic scaffolds within regenerative medicine.

However, even though several promising concepts have been established, some challenges still exist. Our inability to reprogram stem cells into uniform populations with no unwanted potency and to predictably control endogenous cells is restricting the progress of the field [63, 64]. Synthetic biology has the potential to overcome these limitations and could, in future, facilitate true tissue regeneration.

4 Bioprocessing and control

Bioprocessing and its control have come to play a pivotal role in both the synthetic biology and regenerative medicine fields. Especially with the development of large scale bioreactors, cryopreservation of cells [65] and other exciting technologies, such as single-use cellular expansion technologies [66] and serum-free suspension cultures [67], which are discussed in detail elsewhere [66].

Bioprocessing aides the development of biopharmaceuticals, for instance therapeutic proteins, polysaccharides, vaccines and diagnostic tools as well as the development of antibiotics [68]. In the context of regenerative medicine, the cell itself is the final treatment product from the bioprocess rather than the means by which to produce a desired substance or outcome [66]. This means that the product itself is the bioprocess, suggesting that anything and everything within the process could affect the overall outcome of the product, thereby making the bioprocessing of mammalian cells extremely difficult.

This being said, synthetic biology has been shown to improve the overall stability of bioprocessing. This can be achieved by providing an increased robustness during bioreactor cultivations, improved fermentation characteristics and product yields and also the design and application of termination signals [69]. Termination signals are stop signals that are found in the transcribed regions of DNA templates and specify the end of translation of transcription of DNA. With regards to synthetic biology, if the applied termination signal functions successfully, it could become an asset with regards to bioprocessing, but if it fails to effectively terminate transcription, it could lead to major problems in plasmid-based genetic devices. In response to this, Mairhofer *et al.* [69] conducted research into combining a synthetic T7 termination signal with two transcriptional terminators (rrnBT1 and T7) and were able to increase the termination efficiency from the 80% given by the native T7 terminator, to 99%. It is research such as this, which can lead to an increase in the stability of bioprocesses and instil confidence in their use within fields like regenerative medicine, where safety and certainty in the product is of the utmost concern.

Another way in which synthetic biology has aided bioprocessing is through the use of biosensors. Genetically-encoded biosensors, for instance are pieces of DNA that contain the instructions for a biosensor circuit [70]. They have the ability to monitor cellular stresses online and maintain control of cells in large bioreactors, resulting in a gain in productivity whilst ensuring robustness and reproducibility in the overall bioprocess [70]. Although the use of synthetic biosensors shows promising results, there exist several concerns with regard to their safety. Schmidt and Pei [71] state that there is the possibility of misuse, dual use and a risk of biosecurity, they claim that there is a lack of efficient toxicological assessment methods available to evaluate the synthetically derived biosensors. In comparison to this, Bhatia and Chugh [72] state that synthetic biology has the potential to address the biosafety fears surrounding biosensors, and is currently researching into devising 'self-destruction' or tracking strategies within microorganisms. If these strategies are proven successful and their safety is guaranteed, in future, similar systems could be incorporated into mammalian processes.

It is believed, if synthetic biosensors are to progress within research, one of the major challenges must be addressed. That is, that a robust and efficient biosensor must be developed that contains a regulatory pathway that governs use and safety of the product [72].

5 Stem cell therapies

Once synthetic biology was proven to be applicable to eukaryotic cells, the focus began to shift towards adding new tools to the current available technologies. These tools include the modification of signalling pathways related to disease, novel gene level intervention (gene therapy) and cell therapies [73]. Cell therapies are a primary area within regenerative medicine, especially when combined with the use of stem cells. Various stem cell types [including umbilical-cord, embryonic, mesenchymal, adipose, cord blood, limbal and induced pluripotent stem cells (iPSCs)] have already been used in treatments for a myriad of conditions, with some successfully reaching clinical trials [74–76]. Conditions include bone marrow transplantation for leukaemia, other blood diseases such as Fanconi anaemia and various cancers. In addition, blindness through corneal damage or disease such as macular degeneration, bone and cartilage repair, heart attack, stroke and critical limb ischaemia [74, 77].

Some therapies that incorporate stem cells include the healing of burns and wounds [78], and cancer based immunotherapy [79]. With regards to wound healing, stem cell therapy offers a treatment for chronic skin wounds such as diabetic and venous ulcers, as well as deep acute wounds. At this current point in time, none of the technological approaches are able to regenerate skin appendages, most notably hair follicles and sweat glands. In future, by incorporating synthetic biology and the availability of adult stem cells and iPSCs from the patient, there's the possibility of generating these structures without risking immune rejection. This could then potentially form the basis of new therapies that address the current limitations [78] and producing a treatment more beneficial for the patient.

Synthetic biology can also be implemented in cellular engineering and genome editing of host cells, as shown in the cell-based cancer immunotherapy research, where a patient's T cells were engineered and used as therapeutic agents. In this instance, synthetic biology can be utilised to improve the efficacy of cell-based cancer therapeutics, through the development and application of synthetic sensors, switches and circuits [66]. This could potentially reduce the time and cost of manufacturing, thereby making it highly desirable for clinical applications. Chakravarti and Wong [79] claim that by applying synthetic biology, cell-based therapies can become safer and more powerful. As a result of this, in future, both regenerative medicine and the healthcare sector are anticipated to benefit greatly from synthetically modified cells, in that they will have use of cells that have the ability to discriminate between cell states, integrate multiple inputs and respond appropriately to combat diseases and injuries. It is thought that with improved tools and well-characterised bio-parts, synthetic biology has the potential to revolutionise therapeutic research and increase our understanding of disease progression [80]. Potentially

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allowing synthetic biologists to overcome many if not all of the obstacles faced by stem cell therapies and advance the field towards clinical applications.

6 Gene therapies

With synthetic biology's remarkable ability to construct functional biological devices from well-characterised components, it has since become possible to engineer synthetic control networks and signalling cascades that can program cell morphology [81], metabolic behaviour [82, 83] and therapeutic interventions [84] with high precision [85]. By applying synthetic therapeutic gene circuits in mammalian cells to control specific signalling networks, it presents the possibility of improved therapeutic treatments for numerous diseases and disorders [85]. These range from metabolic disorders such as obesity [86], Type 1 [87] and Type 2 [86] diabetes, to cancer [88] and immunological diseases.

A significant challenge faced by synthetic biology is to integrate these synthetic circuits into the desired location within a host genome, whilst maintaining the genetic integrity of both the circuit and the host [80]. The CRISPR-Cas9 system [89], derived from a bacterial defence mechanism, is a breakthrough genome editing technology that has guided synthetic biologists to take steps towards accomplishing this. With its use, researchers can introduce correct mutations into the genomes of cells and organisms with a level of ease and proficiency that was not previously possible [90]. It is because of this that CRISPR-Cas9 offers unparalleled opportunities in fighting genetic disease, the targeted modification of whole genomes, *in vivo* models for drug discovery [88] as well in cell therapy and regenerative medicine [90, 91]. Given the rapid pace of the fields development, the technology is continuously adapting and improving. Current advancements continue to make its use easier and faster, thereby yielding significant benefits for both preclinical and clinical use.

Despite this, although CRISPR-Cas9 is considered the gold standard technology, especially when compared to other genome-editing technologies [92, 93], it too experiences some limitations with regards to greater understanding of off-target effects and optimisation in application that must be dealt with before being viable for altering the human germline for preventive purposes. Heidari *et al.* [91] state that these limitations are brought on by our limited understanding of genetics. As such, if technologies such as CRISPR are to reach their full potential and be translated to the clinic, we must first gain a better understanding of genetic disorders, particularly those involving multiple genes and the genetic interventions required, and then find methods to surpass the pre-existing translational barriers surrounding gene therapies. Current translational barriers centre primarily upon scale-up of manufacture as well as quantity and quality of donor samples for therapeutic targeting in some instances. With the advances of DNA foundries and continuing improvements in efficiency and scale for developing synthetic tools and more accessible DNA synthesis, synthetic biology has the potential to offer novel manufacturing solutions that cross-over to overcome translational barriers in other emerging fields such as gene therapies in the near future.

7 Current challenges

Stem cell research has always experienced ethical, legal, and social complications [94, 95]. When synthetic biology is added to that, you then include the issue of using genetic modification, which in itself has its own set of social and ethical problems. As a result of this, when a cell that is used in regenerative medicine is genetically modified, it will be considered a new product. This suggests that before gaining approval from various health boards, for instance the European Medicines Agency (EMA) or the US FDA, the product will have to undergo another round of extensive characterisation to prove that no other region in its genome has been altered. Consequently, in addition to placing genetic burden on the cell, it will also have to undergo extra physical stress, which can greatly affect the phenotype of the cell.

Having said this, guaranteeing safety and gaining public approval are some of the major challenges faced by synthetic biology today. As such, extensive characterisation is just one way of ensuring confidence in the end product. Another way of combating the challenges faced is by finding alternative methods for treatments. For example, one of the ongoing issues faced within regenerative medicine is the use of embryonic stem cells (ESCs) and their derivation from human embryonic blastocysts. Research has been conducted to consider the use of induced pluripotent stem cells (iPSCs) as an alternative to ESCs, as they overcome two of the ESCs primary problems; immune rejection following transplantation and ethical concerns as they are derived from adult cells [64, 78]. Having said this, using iPSCs also presents insurmountable risk, especially when it entails the genetic modification of both donor and host cells, generated by the transfection of a viral vector following iPSC transplantation [44, 96], which has parallels with further application of synthetic biology in the field. It is for this reason, that entry into the clinic is slow as much work must be done to guarantee the safety of their use as iPSCs are being incorporated into preclinical and clinical applications, such as for age-related macular degeneration (AMD) [76].

Another key aspect of synthetic biology is to guarantee reproducibility of systems across various applications and cell types, thereby improving its engineering and translational capability. Currently, when the circuits are introduced across different organisms and cell lines, synthetic biology experiences complications with variable circuit efficacy. This could potentially be overcome by focusing on the use of standardised circuit elements that have already been proven to function in multiple bacterial or mammalian hosts. Doing this could increase the ease of manufacturing as well as ensure that therapies reach patients much faster [80].

8 Conclusions

Synthetic biology is a field that is still in its infancy, but is continuously expanding into new and exciting areas of research. It has already aided the development of a myriad of industrially and pharmaceutically useful compounds, devices and therapies and is now helping the field of regenerative medicine to progress. Tissue engineering, materials and scaffolds development, bioprocessing control, stem cell and gene therapies are all areas that have greatly benefited from its contribution, and are all areas in which synthetic biology can truly make a difference in future.

Despite its many achievements, it still faces several challenges which many researchers are continuously trying to overcome. As synthetic biology continues to advance, there are still numerous avenues it could pursue within regenerative medicine. One such avenue is to implement synthetic biology more into mammalian cells, making the therapy or product a step closer to being clinically viable. This would greatly benefit the emerging healthcare industry, where mammalian products are severely lacking. Another avenue that could be explored in future is the use of synthetic biosensors to improve the manufacturing process of gene therapies. An innovation such as this would help researchers meet future clinical demand for gene therapies, whilst still maintaining the natural phenotype and efficacy of the cells.

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