Improvement of Biomedical Structural Polymers by Synthetic Biology Methods

Vyacheslav Gennad'evich Malyutin¹*, Valentina Olegovna Sevagina², Viktor Anatolevich Kokotov³, Vitaly V. Goncharov⁴, Alexander Markov⁵ and Karina Nikolaevna Shalneva¹

¹St. Petersburg State Pediatric Medical University, Street litovskaya 2, 194353, Russia.
²First Pavlov State Medical University of St. Petersburg, Russian Federation, Russia.
³I.M. Sechenov First Moscow State Medical University (Sechenov University), Russia.
⁴Kuban State Agrarian University (Named after I. T. Trubilin), Russia.
⁵Tyumen State Medical University, Tyumen, Russian Federation, Russia.

Authors’ contributions

This work was carried out in collaboration among all authors. Authors VGM and VOS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author VAK and VVG managed the analyses of the study. Author AM and KNS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Materials that have a biological origin find many applications in both Biomedicine and other Sciences. However, the properties of these materials are difficult to change, since natural biosynthetic mechanisms are difficult to explain, simulate, or adjust. Thus, many materials of biological origin are isolated from natural tissues or their substitutes are produced recombinantly, and then modified in the process of experimental application.

A major shift in this paradigm is caused by the emerging field of synthetic biology, which introduces innovations in the "tool kit" of methods for tuning biomolecules and biosynthetic mechanisms. Relative to materials, this led to higher product titers due to reprogrammed natural biosynthesis and allowed the development of new materials by combining the desired domains. Here we can focus

*Corresponding author: E-mail: a1ewhite@yandex.ru;
on recent applications of synthetic biology to bio-derived ribosomal and non-ribosomal polymer materials for biomedical applications. It is also interesting to describe modern methods that will affect the production and design of biomaterials in the near future. Continuous innovation at the intersection of synthetic biology and materials science promises to usher in a new era of biomaterial design and synthesis.

Keywords: Biomaterials; synthesis; biomolecules; structural polymers.

1. INTRODUCTION

Structural materials of biological origin, such as proteins, polysaccharides, and polyesters, are central to the field of biomedical engineering-tissue engineering [1]. The biosynthesis of these materials involves the processes of gene expression, metabolism, cellular signaling, and extracellular installation. The complexity of these processes has historically been an obstacle to controlling the properties of materials in vivo, even after the advent of recombinant DNA technology. This limits the range, diversity, and function of functional materials of biological origin. To overcome this limitation, we need tools that can create biological processes [2].

Innovations in the emerging field of engineering, called synthetic biology, have created tools that allow one to design, predict and control complex biological processes, removing historical obstacles to creating materials in vivo. Thus, the application of the "tool box" of synthetic biology to materials of biological origin marks a new era in which materials of biological origin are reproduced, imitated and modified using engineering biosynthesis [3].

According to the above, it is necessary to clarify recent studies of the application of synthetic biology to structural biomolecules. It is also advisable to study the current state of synthetic biology and its significance for the synthesis of materials [4]. Given its potential, we can conclude that synthetic biology is the key to the extensible production of structural biomaterials with the desired properties.

2. BIOMEDICAL STRUCTURAL POLYMERS

Biomedical structural polymers produced by microbes can be improved or modified using synthetic biology. These biomaterials are divided into two broad classes – proteins synthesized by ribosomes and polymers synthesized by non-ribosomes. Structural biomaterials based on ribosomal proteins mainly consist of polypeptides that make up the extracellular matrix (ECM) or imitate it [5]. These include ECM proteins such as collagen, laminin, fibronectin, and elastin, which are currently obtained from tissues or as a result of low-yield recombinant fermentations. For example, collagen has historically been ineffective, and has only recently been produced in tobacco factories in quantities sufficient to produce medical devices based on collagen [6]. In addition, heterotrimeric laminin is commercially available and can be adapted on a laboratory scale.

The limited availability of ECM proteins has resulted in ECM protein simulators being obtained from non-mammals. These include recombinant bacterial collagen-like proteins, arthropod silk, and insect elastomeric elastic. These ECM analogs can be obtained in microbial fermentation systems with recombinant DNA. In particular, silk fibroin is a protein with remarkable mechanical properties, which prompted its widespread use as a structural biomaterial [7].

Structural polymers synthesized by non-ribosomes are naturally formed in several microorganisms and are of interest for biomedical applications and, more broadly, as bioplastics and biofuels. These include exopolysaccharides, polyesters, and other heteropolymers [8].

Exopolysaccharides are structural biopolymers consisting of sugars that are either synthesized extracellularly or secreted by microorganisms [9]. Installation mechanisms allow the formation of high-molecular polysaccharides with various properties, including branched or linear structures and homomeric or heteromeric composition. Several such exopolysaccharides have been identified as candidate molecules for the production of "green" materials, a review elsewhere [10].

Although many polysaccharides are used as structural materials, bacterial cellulose and hyaluronic acid have recently come to the fore. Bacterial cellulose is widely used in electronics, acoustics, and medicine due to its regular nanostructure and high purity [11].
Hyaluronic acid (HA) is widely used in synovial joint supplements, hydrogel formation, and cosmetics. HA is initially synthesized and secreted from as part of the immune capsule. In addition to exopolysaccharides, polyhydroxyalkanoate (PHA) polyesters are produced by microbes as carbon and energy storage materials and are of interest as candidates for biomedical structural frames, as well as a sustainable alternative to petroleum-derived polyesters [12].

Another interesting polymer is the poly-γ-glutamic acid (γ-PGA) heteropolymer, which is linked by amide bonds between the α-amino group and the γ-carboxylic acid group through non-ribosomal synthesis in microbes. gamma-PGA turned out to be a thermosensitive injectable hydrogel for drug delivery [13]. Thus, each of these non-ribosomal polymers has a unique biosynthetic mechanism and is useful as structural biomaterials.

Synthetic biology techniques can control the biosynthesis of each of the materials described above, including gene expression, protein function, metabolism, secretion, and extracellular assembly. Most of these methods focus on the development of gene expression — transcription, translation, and post-translational modifications [14]. In addition, metabolic engineering and protein engineering techniques have been applied to alter metabolism, secretion, and extracellular installation. Below, we highlight these recent works that demonstrate the growing convergence of synthetic biology and structural biomaterials.

Materials are the product of gene expression, metabolism, secretion, and extracellular installing processes. The development of each of these important stages is necessary for precise control of all the properties of biologically based structural materials [15].

Control of transcription, translation, and posttranslational modifications should also be considered. The Central dogma of biology is an important component of biological processes, and synthetic biology seeks to control them with parts and devices. In recent years, research in synthetic biology has focused significant efforts on developing standards for modular molecular cloning so that genetic parts can be assembled in any combination [16]. This has led to standard, characterised collections of parts for many host organisms, including producers of biomaterials such as the cellulose-secreting bacterium Komagataeibacterhaeticus. They are extremely effective in tuning the expression of heterologous genes, and their modular nature makes it possible to quickly create prototypes of combinatorial libraries [17].

In addition to assembling modular parts, the development of individual functional parts remains an active area of research, with recent work describing the following:

- new promoters;
- terminators;
- the binding sites of ribosomes;
- orthogonal polymerases;
- transcription factors [18].

These achievements are further highlighted by the ongoing development of orthogonal programs based on the T7 bacteriophage that function independently of the native cellular apparatus. Advances in the characterization of expression systems based on T7 polymerase and the development of new transcription factors for recruiting T7 RNA polymerase make it possible to reprogram cells without interfering with critical cellular processes [19].

The RiboTite system is potentially related to the synthesis of secreted ribosomal biomaterials. This tool consists of T7 and orthogonal riboswitches, corresponds to the speed of central dogma processes with the frequency of secretion to avoid toxic product accumulation in cells. In this system, transcription is regulated by placing the gene of interest and orthogonal RNA polymerase T7 under the control of inducible promoters [20].

Translation control is achieved using an orthogonal riboswitch capable of sequestering the ribosome binding site. Using components such as RiboTite to control the recombinant synthesis of structural ribosomal biomaterials can increase productivity.

Unlike transcription and translation, posttranslational modifications were harder to create. The study of the involved enzymes, such as oligosaccharyltransferases (OST), is complicated by the fact that they are large, complex, and difficult to overexpress in host cells. To overcome this problem, researchers recently, a cell extract platform has been developed for rapid prototyping of functional, membrane-embedded OSTs to enable characterization and design of
these critical enzymes in a cell-free environment. Such a platform successfully activated glycoprotein production [21].

In this approach, an OST-rich and lipid-bound strain of *Escherichia coli* was created with oligosaccharides, and a cell extract from this strain allowed the combination of extracellular transcription-protein translation with glycosylation. These prospective technologies promise to solve the problems associated with post-translational modifications in the synthesis of complex biomaterials.

Within the framework of this topic, it is advisable to explore approaches to the synthesis of biomaterials based on the methods of metabolic engineering.

Polymers synthesized by non-ribosomes are the end result of an enzymatically controlled biosynthesis pathway and depend on the cell’s metabolic network in contrast to proteins. Thus, the metabolic pathway and Transporter engineering are particularly effective for improving the synthesis of exopolysaccharides, polyester, and heteropolymers [22].

Recently, recombinant production of hyaluronic acid (HA) in *Bacillus subtilis* has been significantly improved through bio-exploration in search of improved transporters and lipid membrane remodeling. In addition, the PHA polyether titers were improved by new gas and liquid feedstocks, transcriptome analysis of highly productive strains, and recombinant production in model organisms. In addition, PGA heteropolymer production was increased by using recombinant hemoglobin to enhance respiration. Most studies emphasize the applicability of metabolic engineering to the synthesis of structural biomaterials, especially non-ribosomal polymers [23].

The process of secretion is also interesting in this respect. Cells modulate their local microenvironment by secreting macromolecules such as proteins and carbohydrates. Depending on the cell, secretion may require the transport of large, possibly charged molecules across the cell membrane, as well as through one or more cell walls.

Synthetic biology methods can use a native cellular transport mechanism to secrete recombinant molecules. In the case of ribosomal biomaterials, proteins can be labeled with specific amino acid sequences so that they can be identified using a secretion apparatus. The secret label libraries of the yeast *Pichia pastoris*, E. coli, and Lactobacillus casei were recently identified and characterized. Significant work has also been done in the development of protein secretion systems themselves, such as the type III secretion system in *Salmonella enterica* [24].

Recently, it has been shown that the regulation of type III secretion genes associated in *S. enterica* enhances the secretion and purity of several recombinant biopolymers forming proteins, including Pro-resilin, TROPO-elastin, and silk [25].

As for non-ribosomal polymers, many of them are secreted as they are synthesized - for example, the exopolysaccharides cellulose and hyaluronic acid. This allows the synthesis of high-molecular polymers that do not need to be transported through the cell membrane after synthesis. These mechanisms have been thoroughly clarified, but only recently. The main problem is the complexity of the mechanism of transmembrane synthesis-secretion. For example, hyaluronic acid can be recombinantly produced in non-pathogenic *B. subtilis* and *Lactococcus lactis*, but only in low-molecular chains. This underscores the need for further work in the field of engineering mechanisms of secretion [26].

Many ribosomal biomaterials are collected extracellularly. Spider silk is widely studied as a basis for tissue engineering, and the applications of synthetic biology to the engineering of silk-based materials are well reviewed in other sources. The main problem with silk materials is the extracellular assembly of large proteins. One recent approach to engineering assembly is a new system with split inteins that secretes subfragments that self-assemble extracellularly. The split intein system involves the use of peptide domains attached to the ends of proteins that self-excite with minimal scarring when joined.

Recently, *Zhang* et al. used this system to provide extracellular assembly of two very large recombinant spider silk spidroins (290 and 282 kDa) into a 556 kDa spider silk product [27].

Researchers studying the underwater adhesion properties of recombinant mussel foot proteins also used the split-intein system to assemble high-molecular oligomers with excellent adhesion.
properties. Other approaches include genetically encoded click chemistry labels that allow spontaneous formation of covalent bonds under physiological conditions between genetically encoded domains or sorting-based assembly [28].

Both methods allow the expression of smaller heterogeneous subunits, which can then be combined into a larger biomaterial. These approaches emphasize the applicability of synthetic biology and protein engineering to create improved functional ribosomal biomaterials.

The vast design space provided by synthetic biology is not limited to the materials that currently exist. For example, new hybrid biomaterials can be produced by combining the biosynthesis of two or more structural biomaterials. In addition, new host organisms with unique biosynthetic capabilities can be domesticated to produce new biomaterials. In addition, genetically encoded logic can be used to control biosynthesis, possibly for structuring and forming complex biomaterials. In the next section, we describe recent advances in creating new structural biomaterials using synthetic biology and highlight the potential of new host organisms and programmable logic to further influence the creation of new bio-derived structural materials [29].

Synthetic biology allows you to synthesize new materials that mimic natural fabrics and materials that have no analogues. One of the first examples is the use of metabolic engineering to synthesize new cellulose-chitin heteropolysaccharides in bacteria. In addition, the desired properties in new materials can be achieved by abstracting functional domains from existing biomaterials and improving or combining them with other domains [30].

Silk-elastin-like protein copolymers have been developed that use both the mechanical strength of silk and the heat-sensitive properties of elastin. This new material combines bacterial CLP with functional domains and fragments of ECM molecules to produce new materials with emerging functionality. The heat-sensitive properties of elastin have also been implicated in the elastin-B-collagen-like peptide copolymer, which allows the formation of self-organizing nanobubbles in response to a heat stimulus. These materials can be functional and dynamically recently programmed cells have been integrated into life, a wearable elastomer hybrid hydrogel. Moreover, light-sensitive elements of biological origin were used to create a gel material that could reversibly capture and release molecules when exposed to light [31].

Finally, protein engineering can create new functional variants of materials of biological origin. For example, multiple non-canonical amino acids have been inserted into elastin-like polypeptides using cell-free synthesis, allowing many potential new properties and sites for modification in ribosomal biomaterials [32].

Synthetic biology efforts actively transfer genetic parts from model organisms such as E. coli to non-domesticated or under-developed recombinant hosts. Now you can select a local producer and develop new tools for synthetic biology to make it genetically controlled. The most successful example of this is the domestication and development of a cellulose-producing K. rhaeticus isolate [33].

Tools are also being improved for long-studied but under-developed recombinant hosts. These include B. subtilis, Arabidopsis, yeast clade CTG, and microalgae Chlamydomonas reinhardtii. Host vectors can be used to create many members of the microbiome, such as the bee’s gut.

As new hosts are domesticated and more effective tools are developed for alternative hosts other than E. coli, the range of choice for the production host will expand. This will lead to new problems when selecting a suitable host or hosts for the production of the biomaterial of interest. However, the advantage is that these new organisms can have unique combinations of growth and biosynthesis mechanisms that can lead to increased production efficiency or provide synthesis of completely new structural materials of biological origin.

In addition to scalable biomaterial production, synthetic biology could provide tools for designing the production of desired materials that would match a specific set of properties on request. Biological organisms are initially able to integrate exogenous chemical, mechanical, thermal, and optical signals along with internal cellular “States” to respond to changes in their environment.

Using synthetic biology, we expanded the range of input signals that cells respond to (for
example, optogenetics) and gained control over how cells respond to these inputs through genetic chains. Given advances in directed hierarchical extracellular Assembly, cells can be reprogrammed to create new materials in response to environmental signals. This can lead to self-healing of biomaterials along with countless other applications of biomaterials controlled by logic [34].

Complex cellular logic is based on inducible genetic switches. With a simple switch, biosynthesis can be turned off during cell growth and turned on during production. A useful method of chemical control is to use inducible promoters that are sensitive to a specific carbon source, especially natural carbon sources such as xylose. The last of these went beyond small molecule inducers to light-controlled butanol synthesis in *Saccharomyces cerevisiae* and temperature-controlled recombinant protein production in *C. reinhardtii* chloroplast turned on unused cold-sensitive tRNAs. These induction signals can provide controlled production of materials on demand without the addition of extraneous chemicals [35].

In addition, more complex genetic schemes have been developed that can control the biosynthesis of complex extracellular materials made up of many components. Advanced circuits can be built from latches that allow you to program sequential logic based on the current input and previous States. In addition, synthetic gene generators have also reached the point where they can capture and store input data from the environment in the form of chain frequency shifts.

In addition to *in vivo* advances, *in silico* tools have been developed to help develop synthetic gene chains, such as the Cello framework. These advances in cellular computing can make possible the sequential synthesis of biomaterial components, complex layered structures, or the synthesis of different materials in response to different stimuli - in other words, the dynamic synthesis of complex biomaterials. However, the lack of combinability and predictability for many of these schemes remains a serious problem, limiting the portability of complex genetic schemes in these advanced applications [36].

As a result of synthesis, structural biomolecules can be reproduced with the desired mechanical and chemical properties with yields sufficient for small studies.

3. CONCLUSION

Recently, the use of synthetic biology tools to control gene expression, protein function, secretion, and extracellular installation has opened up a whole new design space. These advances have laid the groundwork for implementing complex biological design workflows that combine synthetic biology at all stages of the biosynthetic process to create functional biomaterials with high yield, allowing you to imagine a rational design of all aspects of the biomaterial.

Computer-aided design software can identify the metabolic pathways that lead to the production of the intermediates needed to build the specified molecule. Once these pathways are clarified, genetic chains and DNA constructs can be constructed to produce the developed material. This combination of synthetic biology, materials science, and computational biology will enable a unified approach to the development of structural materials based on biological materials.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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