Bacteria Genetic Engineering

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Abstract – Molecular communication holds the promise to enable communication between nanomachines with a view of increasing their functionalities and opening up new possible applications. Due to their biological properties, bacteria have been proposed as a possible information carrier for molecular communication, and the corresponding communication networks are known as bacterial nanonetworks. Individual molecules are to be used as building blocks to develop nanomachines but nanomachines cannot be assembled molecule by molecule with the existing technologies. So in the bio-hybrid approach, the naturally existing biological nanomachines are genetically modified by means of molecular engineering to achieve effective communication in the complex nanonetworks. In this paper, few major reasons for choosing bacteria genetic engineering to aid the encoding process and ways to incorporate information in genome along with the various applications of using bacteria as the information carrier are discussed. The challenges and the current advancements in the field of molecular communication are also analyzed.

Index Terms – Bacteria, BioBricks, Molecular communication, Nanonetworks.

1. INTRODUCTION

Over the years we have observed some significant advancements in the field of communication in which different approaches have been implemented resulting in increasing the throughput and efficiency of a communication network. As of late, huge progressions have been made in controlling and building new materials on the nanometer-length scale, i.e., at the level of particles and atoms. A hefty portion of these nanostructured materials have indicated huge prospect as essential building blocks for versatile, little, and energy efficient gadgets, photonics, magnetics, and electromechanical systems to change computing and communication in the future. These energy efficient gadgets are called Nanomachines.

Nanomachines can be characterized as manufactured or natural nanoscale devices that perform basic tasks, for example, calculation, detecting or actuation. Advancement in natural science and nanotechnology makes it conceivable to build biological nanomachines, alluded to as bio-nanomachines.

As shown in the accompanying figure, different methodologies can be utilized for the development of nanomachines, ranging from the utilization of man-made components to the reuse of biological substances found in nature. These methodologies are grouped into three principle branches, namely, top-down, bottom-up and bio-hybrid. In the top-down approach, nanomachines are created by methods for downscaling current microelectronic and micro-electro-mechanical technologies without atomic level control. In the bottom-up approach, the design of nanomachines is realized from the (self) assembly of molecular components and combined nanomaterials. On the other hand, in a bio-hybrid approach, existing biological components, for example, Deoxyribonucleic acid or DNA strands, antibodies or molecular motors, are combined with man-made nanostructures to create new nanomachines. This paper mainly talks about the bio-hybrid approach and its applications [1].

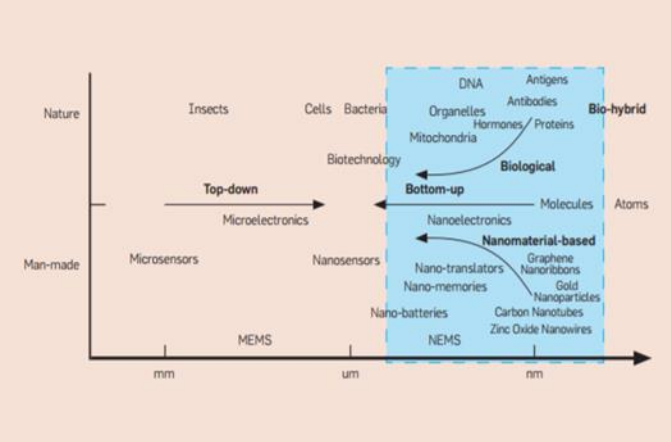

Fig. 1. Approaches for the development of Nanomachines [2]

A nanonetwork or nanoscale network is a set of interconnected nanomachines. Nanonetworks are required to extend the abilities of single nanomachine both regarding complexity and scope of operation by permitting them to arrange, share and fuse data. The abilities and the application scope of these nanomachines emphatically rely upon the path in which they are fabricated.

The capability of interfacing bio-nanomachines has prompted the rising interdisciplinary research region of Molecular Communication. Molecular Communication is characterized as the transmission and gathering of data by means of molecules. The diverse molecular communication systems can be classified according to the type of molecule propagation in walkaway-based, flow-based or diffusion-based communication.

In walkway-based molecular communication, the molecules propagate through pre-characterized pathways by using carrier substances, for example, molecular motors.

In diffusion-based molecular communication, the molecules spread through unconstrained diffusion in a fluidic medium. For this situation, the molecules can be subject exclusively to the laws of diffusion or can likewise be influenced by unpredictable turbulence present in the fluidic medium. Pheromonal communication, when pheromones are discharged into a fluidic medium, for example, air or water, is an example of diffusion based architecture. Different examples of this sort of transport includes calcium signaling among cells, and additionally quorum sensing among bacteria [3].

In flow-based molecular communication, the molecules spread through dispersion in a fluidic medium whose stream and turbulence are guided and predictable. The hormonal molecular communication through circulatory systems inside the human body is a case of this sort of spread. The flow-based proliferation can also be acknowledged by using carrier substances whose movement can be constrained on the average along particular paths, in spite of demonstrating a random component. A good example of this case is given by pheromonal long range molecular communications. This type of molecular communication can also be accomplished by using E. coli bacteria as chemotaxis.

Regarding the approach of using genetic engineering in molecular communication, Bacterial chemotaxis in flow-based molecular communication system is one major approach. While bacterial chemotaxis is employed to transfer data, the major steps of the communication process is as follows:

- *Sensing*: Biological circuits can sense a variety of environmental conditions like light, temperature, and presence of food or poison. At the point when there is a change in the environmental conditions, the rate at which the promoters activate the genes changes resulting in a change in the concentration of proteins.
- *Encoding*: The DNA Processing Unit in the transmitter encodes the message to transfer in a double-stranded DNA molecule.
- *Encapsulation*: The plasmid generated in the encoding step is transferred to a carrier, and the active section of the plasmid is expressed, and the propagation step starts.
- *Propagation*: The carrier swims from transmitter to receiver using bacterial chemotaxis with the carrier as donor and the receiver as acceptor [4].
- *Decapsulation*: After receiving the message, the receiver kills the bacterium, which would try to redeliver the message otherwise.
- *Decoding*: In this step, the receiver uses its DPU to sequence the message region of the plasmid. Sequencing is the process of determining the primary structure, i.e., the sequence of nucleotides, of a DNA strand. Then the message can be decoded and processed at the receiver. This step concludes the communication process.

In this paper, we discuss that why bacteria is the ideal biological organism for Molecular communication and how we genetically modify it to transform it into an ideal information carrier.

The remainder of this paper is organized as follows. In Section II, we present the concept of refining the genome of an organism according to our needs and also talk about the reasons as to why we choose bacteria as the carrier. We present various approaches used in the field of Synthetic Biology to genetically alter the DNA of a bacteria. Starting with BioBricks, iGEM Competition and CELLO Software developed to design computational circuits in living cells. In Section III, we explore various fields such as Biomedical, environment and manufacturing where the concept of Molecular Communication can be applied. Section IV and Section V talks about the current scenario and some of the challenges faced by the Molecular Communication industry. We conclude the paper in Section VI.

2. BACTERIA GENETIC ENGINEERING

Apart from getting benefitted by the existing form of biological organisms around us, the technological advancements in the field of Biotechnology has paved possibilities to the modification the genome of organisms to cater the needs. To decide on the type of organism and how to alter the genome for a specific need, we should get to understand the basics of biological organisms and the way the genetic material is carried upon from parent to offsprings.

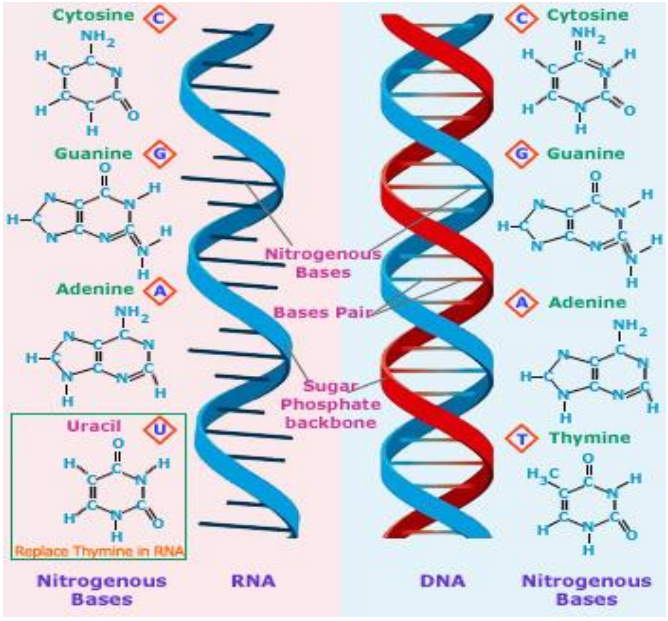

Fig. 2. DNA and RNA structure [5]

Nucleotides, which are a combination of five carbon sugar structure along with one nitrogenous base and one phosphate group, forms the basis of a gene. The nitrogenous base can be of either purine or pyrimidine base. All the genetic components can be represented with two of the purine bases i.e., Adenine and Guanine and three of the pyrimidine bases i.e., Cytosine, Uracil and Thymine. Generally, in chromosome structure two strands of DNA are bound by alternate bondings. Adenine and Thymine forms two hydrogen bonds while three hydrogen bonds are formed between Guanine and Cytosine with Uracil replacing Thymine in RNA. A particular sequence of combination forms a gene which gets the responsibility of a particular function. Many sets of gene form a DNA strand and furthermore continuous double helical DNA strands along with small proteins called histones form the basic set of chromosomes. Being the genetic material, DNA carries the genetic information of an organism. The genetic information present in the DNA is transcribed to messenger RNA known as mRNA which in turn with other RNA's like tRNA and rRNA aids the process of translation to produce the desired proteins i.e., amino acids [6],[7],[8].

Apart from chromosomes, DNA is present in plasmids. Plasmids are circular and are present mostly in all bacteria. Plasmids lack histone protein and doesn't transcribe to genetic functions. Plasmids provide genetic advantages like antibiotic resistance and an exact replica of plasmid is transferred to daughter bacteria through conjugation. The DNA in plasmids mainly constitutes for the survival of the bacteria and it can encode a protein that offers selective advantage for the host [9]. Plasmids, in some cases, can be integrated into the host bacteria as a new plasmid and thus replication produces bacteria with new plasmids or in some other cases, it can be integrated into the bacterial chromosome sequence.

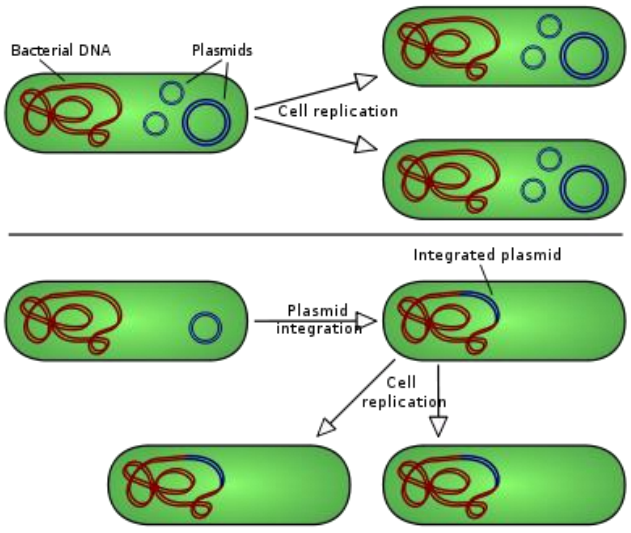

Fig 3. Plasmid in Bacteria

Different regions of plasmid can be classified as *Transfer region*, *Routing region* and *Messaging region* [10], [11].

- *Transfer region* - contains information about vital process like replication, genetic traits.
- *Routing region* - genes to aid the difference between message laden bacteria and the empty bacteria.
- *Messaging region* - message along with the transmission and destination network details.

A. Why Bacteria?

Presence of nucleus and diploid set of chromosomes render the Eukaryotes too complex compared to haploid prokaryotes like Bacteria. For genetic modification of an organism, modification has to be done in the genetic material of every cell [12]. Thus naturally the prokaryotes become the best choice for genetic modification. Also in case of bacterial cells, plasmids can be easily removed and in vitro operations like inserting or deleting the specific sequences of DNA can be performed.

And another major factor in genetic engineering is the need for quicker reproduction of the species under study enabling the scientists to learn the aspects of new traits passed on from parents to offsprings [12]. Exponential reproduction of bacteria qualifies it for this trait.

B. How to Program the Bacteria?

In the next part of our paper, various techniques which are used for the process of synthetic biology are presented. We talk about various programming languages used to rapidly design complex, DNA-encoded circuits that give new functions to living cells.

Synthetic Biology: It is the design and construction of new biological elements, for example, enzymes, hereditary circuits, and cells or the redesign of existing biological frameworks. Synthetic biology expands on the advances in molecular, cell, and systems biology and tries to change biology similarly that synthesis changed chemistry and integrated circuit configuration transformed computing. The component that distinguishes synthetic biology from traditional molecular and cellular biology is the focus on the design and development of core components (parts of enzymes, hereditary circuits, metabolic pathways, and so on.) that can be demonstrated, understood, and tuned to meet particular performance criteria, and the assembly of these smaller parts and devices into larger integrated frameworks to solve specific issues.

C. BioBricks

BioBricks is a programming language for Synthetic Biology. BioBrick standard organic parts are DNA arrangements of characterized structure and function; they share a typical interface and are intended to be formed and consolidated into

living cells, for example, E. coli to build new organic frameworks [13]. BioBrick parts represent an effort to present the engineering principles of reflection and standardization into synthetic biology. The trademarked words BioBrick and BioBricks are accurately utilized as adjectives and refer to a particular "brand" of open source hereditary parts as characterized through an open specialized standards setting process that is driven by the BioBricks Foundation. One of the objectives of the BioBricks venture is to give a workable way to deal with nanotechnology utilizing biological life forms. Another, all the more long term objective is to deliver an engineered living creature from standard parts that are completely understood.

BioBricks starts with characterizing particular sequences named basic parts. These basic parts are flanked by a standard limitation enzyme-based polylinker in a proper plasmid. In this way, a BioBrick fundamental part is a DNA sequence [14]. It's a calculated beast, not a physical thing. A physical plasmid containing the BioBrick sequence is only a plasmid. In this way, a fundamental part is much similar to a word in normal language. It's a string of letters (A,T,C, or G) while English words have 26 letter choices. Like English words, BioBricks are collected into bigger sentences (called devices) in a linear order. Basic parts alone rarely give any movement to a cell similarly as words alone present little meaning. BioBrick essential parts just give action in a cell when other appropriate parts flank the part.

Just as English follows a hierarchical organization of letters > words > sentences > sections > books > libraries, BioBricks take after a progression of base pairs > basic parts > composite parts > systems > ecosystems. The dialect and significant portrayals of this hierarchy are as yet being developed in the SB field, however for the present, all DNAs are either basic parts or composite parts.

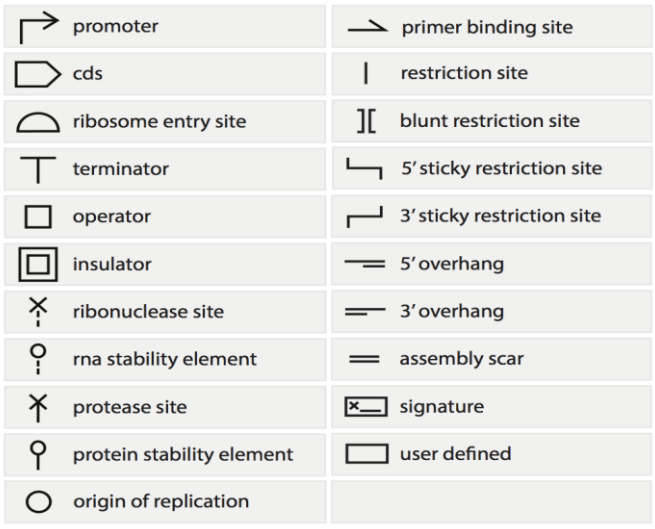

Fig. 4. visual symbols for use with BioBricks standard [15].

There are three levels of BioBrick parts: "parts", "devices" and "systems". "Parts" [13] are the building pieces and encode fundamental biological functions, (for example, encoding a specific protein, or giving a promoter to give RNA polymerase bind and initiate transcription of downstream sequences); "devices" are accumulations of parts that execute some human-defined function, (for example, a riboregulator producing a fluorescent protein whenever the environment contains a certain chemical); "systems" perform high-level tasks, (for example, oscillating between two colors at a predefined frequency).

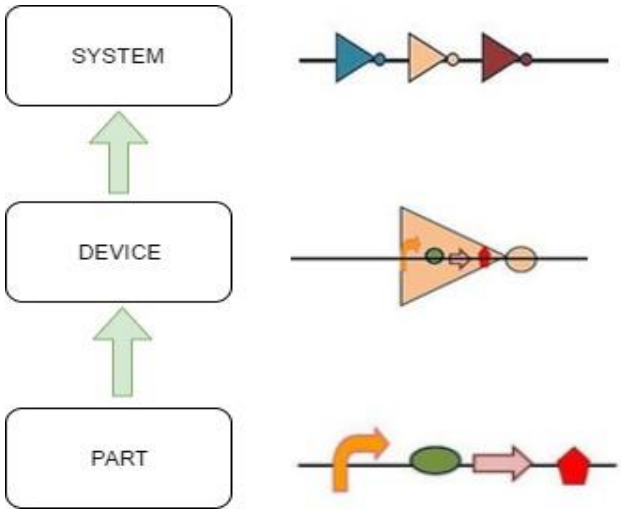

Fig. 5. BioBrick Abstraction Hierarchy

BioBrick Nomenclature:

A new language has been created to explain BioBrick parts [16]. BioBrick vectors are named as pSB#X#. The BioBrick acronym can be broken down as such: the pSB stands for plasmid synthetic biology, the X is the antibiotic resistance gene, the first number sign represents the origin of replication, and the second number sign represents the version of the vector, as the vectors are continually being adjusted and updated for better utilization.

The nomenclature of BioBrick parts is typically seen in the form of BBa_E0840 and represents the GFP insert used in the work of this paper. The BBa stands for BioBrick alpha as the alpha stands for the version of the BioBrick. The alphabetic letter following BBa_ has a variety of indications including promoters, protein generators, and primers as the E in this example represents a reporter part (Table 4). The series of numbers following the letter or letters represents a block of numbers set aside for specific groups or teams in the iGEM association. So, in the BBa_E0840 example, the numbers 0840 represent the block of numbers set aside for the Endy lab at MIT, and the 0040 grouping represents a BioBrick extracted from the GFP source brick BBa_E0040, which also includes

the protein and DNA specifics used to create the basic GFP insert in this series of BioBricks.

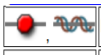	BBa_B... = Generic basic parts such as Terminators, DNA, and Ribosome Binding Sites
	BBa_C... = Protein coding parts
	BBa_E... = Reporter parts
	BBa_F... = Signaling parts
	BBa_G... = Primer parts
N/A	BBa_I... = IAP 2003, 2004 project parts
N/A	BBa_J... = iGEM project parts
	BBa_M... = Tag parts
	BBa_P... = Protein Generator parts
	BBa_Q... = Inverter parts
	BBa_R... = Regulatory parts
	BBa_S... = Intermediate parts
	BBa_V... = Cell strain parts

Table. 1. The letter following the BBa_ sequence in the acronym labeling the BioBrick inserts [17].

BioBrick Projects:

There have been a several inspiring BioBrick projects that not just empower the field of synthetic biology but also give credibility to the rising order and the iGEM competition. The bactoblood project, the immunobrick project, and the odor-producing projects are among a few BioBrick champions that exemplify what can be done with the BioBricks as well as what the spirit of competition can bring out in students involved in the iGEM competition [13], [18].

Among the first BioBricks created were the odor-producing bricks that empowered E. coli cells to produce scents, for example, bananas, flowers, or spearmint. A chemical is displayed to the E. coli cell with the BioBrick present and working, the cell takes up the chemical and changes it into another chemical, in this way delivering the desired fragrance. The winter green BioBrick, or spearmint odor-producing BioBrick, transforms salicylic acid into methyl salicylate, which produces the spearmint odor as the E. coli cells multiply in the culture. The MIT team created this BioBrick in 2006 by harnessing the gene that is able to convert salicylic acid to methyl salicylate [18].

The 2008 Slovenia team created a BioBrick that could identify and destroy the microbes Helicobacter pylori, a typical reason for stomach illnesses. The Slovenia group set going to free the group of Helicobacter pylori using two stages, imitating the infectious bacteria to discover how they are infectious and creating a DNA-based antibody based on the antigenic proteins [18].

The group set about to create a DNA-based immunization from the BioBricks that could be embedded into the cells of an animal, initiate the adaptive immune system in response to the new invading proteins made by the BioBrick, and be sensitive to the infectious proteins of Helicobacter pylori, thereby mounting an immune response to any infectious Helicobacter pylori in the body[13],[14],[18].

Issues with BioBricks:

Synthetic biology encourages the formation of new organic frameworks and devices yet additionally helps in the study of natural biological frameworks. It is not as simple as connecting known genes together in a host cell to change the cell's characteristic programming on the grounds that there are a variety of inherent issues that can hinder the new functionality of the host cell. Problems like background noise (circuitry stability inside the cell), unanticipated protein interaction, metabolic communications, mutation, and evolution can prevent the changing of the cell's natural purpose. Controlling a cell to demonstrate to us the base of such issues may introduce solutions through continued experimentation utilizing synthetic biology. By addressing to one issue at a time and by inspecting some of the successful BioBrick [18] ventures, future complexities can be examined for objective explanations and solutions as the discipline keeps on developing.

D. iGEM

The introduction of BioBricks is an attempt to standardize synthetic biology and make synthetic biology projects reproducible around the globe. The iGEM group (International Genetically Engineered Machines Competition) has made a niche for BioBricks [18] by making a competition to make novel biological devices and frameworks utilizing BioBricks. The utilization of BioBricks can begin to build up some of the main goals of synthetic biology, for example, reproducing natural bacteria to simplify existing functions or make new ones, characterizing the minimal requirements of life through modifying biological systems, creating minimal genomes (protocells) to define the negligible construct of an entity, and gathering orthogonal biological systems prepared to do totally new procedures and in addition retaining the ability to be utilized by the biological system they were made from.

Beyond just building biological systems, more extensive objectives of iGEM includes [19]:

- To enable the systematic engineering of biology.
- To advance the open and transparent improvement of instruments for engineering biology.
- And to help build a society that can profitably and securely apply biological innovation.

iGEM's dual aspects of self-association and inventive control of hereditary material have shown another approach to arouse

student enthusiasm for present day biology and to build up their free learning abilities.

What is iGEM?

The iGEM competition was opened to undergrad and graduate students to encourage the building of a hereditary library, increase enthusiasm for the field of synthetic biology, and raise the acknowledgment of the standardization of biological components, for example, plasmids, enzymes, and host cells. The competition is held by the iGEM establishment, which works through MIT and is in charge of plate distribution and group association, and additionally a jamboree toward the finish of every year to decide the winner of several competitions. The aim of the challenge is to build up a novel biological system, device, or module that can become a part of the always developing library of plasmid inserts in classifications going from the best real world application to the best poster presentation. Each group is given a 384-well plate that incorporates the fundamental segments to make another BioBrick and also the plasmid inserts of past winners.

BioBrick parts fall under a several categories including promoters, ribosome binding sites (RBS), reporters (GFP, RFP, and so forth.), terminators, plasmid backbones, and primers. The BioBricks can likewise be searched by function, and this additionally gives some understanding into what has already been done with BioBrick parts. A portion of the functions that have been produced using BioBricks include: chemotaxis, odorous cells, Coliroid (BioBrick parts required in taking a cell "photo"), biosynthesis, and cell death.

Growth and recent years:

iGEM developed out of student projects led amid MIT's Independent Activities Periods in 2003 and 2004. Later in 2004, a competition with five groups from different schools was held. In 2005, teams from outside the United States partook for the first time. Since then iGEM has kept on growing, with 130 groups entering in 2010. [18]

In light of this expanding size, in the years 2011 - 2013 the competition was part into three locales: Europe, the Americas, and Asia (however groups from Africa and Australia likewise via "Europe" and "Asia" respectively). Regional celebrations happened amid October; and some subset of groups going to those events were chosen to progress to the World Championship at MIT in November

E: Cello Software

As Synthetic biology techniques turn out to be all the more effective, researchers are suspecting a future in which the design of biological circuits will be similar to the design of integrated circuits in gadgets. Cello is a system that portrays what is basically a programming language to design computational circuits in living cells [20], [21],[22]. The circuits created on plasmids expressed in E coli required watchful protection from their genetic context, however primarily worked as specified. The circuits could, for instance, direct cellular functions in response to numerous environmental signals. Such a technique can encourage the advancement of more complex circuits by genetic engineering.

Cello converts electronic design specifications of combinational logic to finish DNA sequences encoding transcriptional logic circuits that can be executed in bacterial cells. A database of transcriptional repressors portrayed in the Voigt lab gives genetic NOT gates and NOR gates that can be created into any logic work.

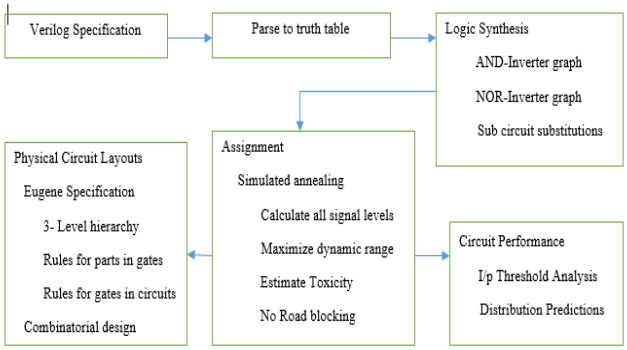

Fig. 6. Cello's basic block diagram [23].

The Cello information is a high-level logic specification written in Verilog, a hardware description language. The code is parsed to create a truth table, and logic synthesis generates a circuit chart with the genetically accessible gate types to execute the truth table. The gates in the circuit are assigned using experimentally described genetic gates. In task, anticipated circuit scores are gathered using Monte Carlo simulated annealing search. The task with the highest score is picked, and this task can be physically executed in a combinatorial number of different designs. The Eugene language [22], a DNA sequence creation language, is used to control constrained combinatorial circuits.

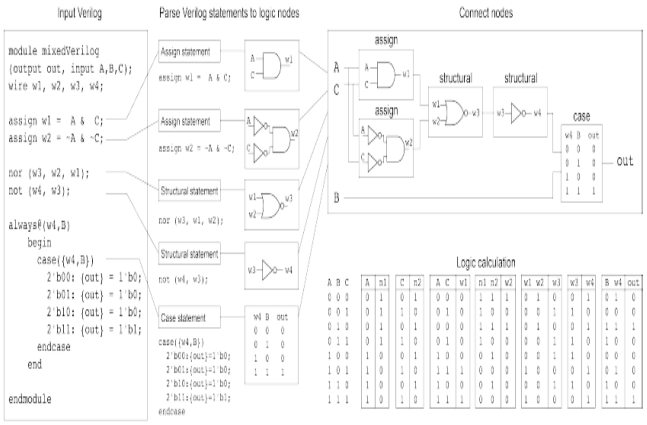

Fig. 7. Process from Verilog code to final genetic circuit [22].

A Verilog file is parsed into individual assign, structural, and case statements. Every statement is converted into a logic node, which can contain at least one gate, or a truth table. Logic nodes are associated by coordinating input-output wire names, and Boolean logic is propagated through every node to figure truth table of the circuit output [22].

Key features [23]:

- Produces a Boolean circuit as a Directed Acyclic graph from a HDL specification (Verilog).
- Utilizes a database of second-generation genetic logic gates whose transfer functions are protected from promoter context.
- Permits clients to upload custom User Constraint File (UCF) documents.
- Scans for the ideal assignment of transcriptional repressors to NOT/NOR gates by signal matching with the experimentally measured transfer functions.
- Offers an extensive variety of assignment algorithms including Simulated annealing, Hill climbing, Breadth first search, Random permutations, etc.
- Creates histograms for anticipated gate REU for each row of the truth table for the best genetic circuit assignment.
- Creates different plasmid versions using the Eugene language for constrained combinatorial list of transcriptional unit requests and orientations.
- Permits outside applications to connect with Cello using a REST API.

3. APPLICATIONS

Applications of molecular communication using nanomachines finds it way into several different areas like biomedical, environmental, and manufacturing [24]. Here we talk about some of the major applications:

- *Health monitoring*: Monitoring performed within an organism like human, animal, or plant enables recognition of specific molecules in the body. The presence of specific molecules may serve as an indication for a disease or a specific medical condition [25] [26]. More detailed information can be used to provide information for further diagnosis. For such applications, nanomachines can be implanted in the body, and molecular communication provides potential techniques for gathering of information about the molecules of the body, conglomerating the information, and transmitting it to external devices.
  Simple synthetic transcription circuitries, constructed by combining bacterial and viral protein domains, have been produced that permit to screen potential medications for tuberculosis or HIV infection. In contrast, more complex gene-expression regulatory circuits have been designed that can detect disease-associated molecular changes in cells like cancer cells and activate cell-death pathways to eliminate the diseased cells. Furthermore, prosthetic devices can be built consisting of transplantable, microencapsulated animal cells equipped by synthetic gene circuits.
- *Drug delivery*: Drug delivery systems encourage the administration and distribution of drugs within an organism. Implanted nanomachines can either use molecular signals within the organism, or released by other nanomachines, to pinpoint the target areas for drug delivery [27] . Existing procedure includes the use of capsules that release drugs in response to specific conditions like temperature. Molecular communication may provide alternative methods to control the release of drugs such as cooperative drug release by a group of nanomachines. lant-derived drugs like artemisinin, which is effective against multi-resistant types of malaria in combination therapy, or for one of the most important cancer drugs, taxol, can be produced in yeast and *E. coli*, respectively.
- *Regenerative medicine*: Nanomachines made of living cells can divide and grow to form a functional structures like organs and tissues. These nanomachines can be applied to aspects of regenerative medicine. As in developmental biology, the formation of a functional structure would advance based on molecular communication among nanomachines. Molecular communication provides strategies to control patterns of communication and accordingly influence the growth and separation of the nanomachines into specific structures [28].

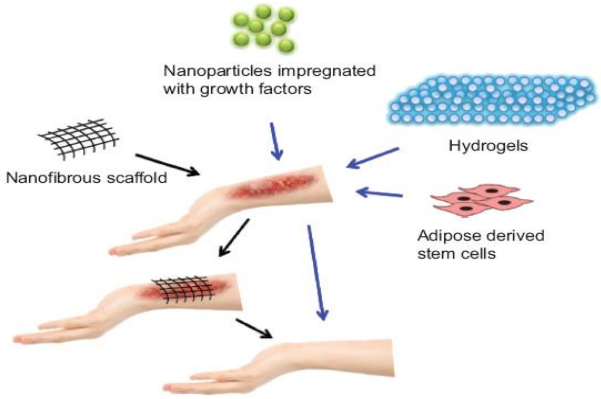

Fig 8. Nanotechnology-based methods for skin regeneration

Skin is more prone to mechanical injuries and infection since it is the outermost covering of the body. Presently, no

bioengineered skin models exist that can completely mimic the anatomical, physiological, and biological characteristics of normal, healthy skin. Wound healing using nanotechnology is a major area of research interest worldwide. The different techniques for skin regeneration includes Nanostructured scaffolds, Nanoparticles as delivery systems, hydrogels and stem cells [29].
Protein fibers, like collagen, fibronectin, and keratin, in the dermis of skin are responsible for cell proliferation and migration. Development of skin substitutes with electrospun nanofibers, that have shown uniform attachment to the surface of the wound without causing an accumulation of fluid. Nanostructured scaffold has shown increased wound healing properties by significantly accelerating cell proliferation and cell adhesion.
Skin regeneration involves attraction of cells and their migration, proliferation of keratinocytes/fibroblasts, and angiogenesis to accelerate these processes. Nanotechnology helps in delivering the growth factors like Platelet-derived growth factor and epidermal growth factor effectively at the target site and also extends local availability considerably, thereby reducing the healing time.

- *Environment monitoring*: The environment might be exposed to poisonous or radioactive agents. Information about these molecules could distinguish problems and guide a map to tidying up the environment in response to illicit pollution or an accidental spill. Nanomachines can be integrated into large or microscale environments to map out the locations of molecules within that environment [30]. Molecular communication provides techniques for the nanomachines to process information from the environment and communicate this information to other nanomachines.

4. CURRENT SCENARIO

Using biological molecules in communication technology, to be specific molecular communication, has first appeared in 2005 and a line of research from that point forward has begun to address different issues on molecular communication. Molecular communication speaks to an interdisciplinary research range that ranges over information technology, biology and nanotechnology, and all things considered information scientists, biologists and nanotechnologists commonly form a collaborative research group. Right now, analysis resolutions are being made at National Institute of Information and Communications Technology (Japan), NTT DoCoMo (Japan), Tokyo University (Japan), Tokyo Medical and Dental University (Japan), Nara Institute of Science and Technology (Japan), University of California, Irvine (USA), Georgia Institute of Technology (USA), York University (Canada), Waterford Institute of Technology (Ireland), Middle East Institute of Technology (Turkey), and numerous others. The first worldwide symposium on molecular communication technology was held in Tokyo 2006 bolstered by National Institute of Information and Communications Technology, Japan. A workshop on molecular communication was conducted in Washington, DC 2008 monetarily funded by NSF (National Science Foundation, USA). Furthermore, technical sessions and board discussions were held at different universal gatherings including IEEE INFOCOM 2005 (International Conference on Computer Communications), BIONETICS [31] 2006 (Bio Inspired models of Network, Information and Computing Systems), and IEEE CCW 2007 (Computer Communications Workshop). Presently, BIONETICS and Nano-Net [26] are the two noteworthy gatherings that accumulate molecular communication researchers.

5. CHALLENGES

Recent research in molecular communication stays limited to the design and analysis of small-scale networks of several bio-nanomachines with simplistic presumptions about bionanomachines and the environment. A key challenge to progress the territory of molecular communication to the next stage is to create powerful and adaptable strategies to make large scale systems which work in the environment of practical applications. In this section, we discuss from a computer networks perspective the research issues and challenges that need to be addressed to achieve robust and large-scale molecular communication networks. [24]

- *Physical Layer*: Some of the major physical layer challenges includes
    - Signal Propagation - Majority of the studies focus on random walk models and diffusion-based models. Increasing their applicability for more complex geometry like intracellular environment and human body are still to be studied.
    - Signal Modulation - Until now, the efforts are based on assumptions like precise transmission and reception of molecule by bio-nanomachine, or synchronized trans-receivers. For enhanced feasibility of modulation schemes and compatibility with bio-nanomachine design, future works without the above mentioned assumptions has to be carried out.
    - Signal amplification - In molecular communication, which depends on propagation of molecules in aqueous environment, attenuation and distortion of signals increases in a nonlinear order. And, so far, there are only limited number of studies on signal amplification and various issues are still to be analyzed.

- Channel capacity - Investigations have to be change focus from theoretical propagation models to computing the channel capacity using physically realistic methods.

- *Link Layer*: Includes but not limited to
  - Error Handling - Though, to detect errors, information molecules with redundant information has been widely proposed, current research has to go a long way to design and compare biologically implementable error handling schemes
  - Addressing - Research so far conducted has suggested techniques to identify the receiver by type of molecule or by location. With these techniques, it's still unclear to determine the address requirements for different network and feasibility of dynamic addressing.
  - Media access control - Handling multiple bio-nanomachines in shared media is yet to be addressed.
  - Synchronization - Synchronization with unknown distance between sender and receiver, with multiple senders, or with non-diffusion techniques are to be further developed.

- *Network Layer*: Mainly includes Routing and In-network processing issues.
  - With bio-nanomachine constantly moving, the problem of dynamically implementing routing procedure is yet to be solved
  - Owing to the limited research in physical and link layer, network layer issues haven't gotten much importance.[24]

6. CONCLUSION

Molecular communication incorporates strategies from biology to collaborate with biological frameworks, from nanotechnology to enable nanoscale and microscale interactions, and from computer science to coordinate into bigger scale information and communication processing systems. In spite of the fact that research in molecular communication is in its earliest stages and has just duplicated functionality already available in biological systems, proceeding with research in molecular communication will lead to coordinated molecular communication systems in which different components of it will cooperate to give full communication functionality. Molecular communication has critical potential, since it interfaces straightforwardly with biological systems at nanoscales and microscales, and it might conceivably affect different technological domains including health sector e.g., nanomedicine [32] and tissue engineering [33], environment sector e.g., environmental monitoring, IT sector e.g., unconventional computing and body sensor networks [34], and military applications e.g., biochemical sensors.

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