OPEN ACCESS Freely available online

Advancement in Genetic Engineering

Review Article

Genetically Engineered Biomaterials

SinithRashmin Withanage*, Kladko Daniil

ITMO University, SCAMT Laboratory, Saint Petersburg, 191002, Russian Federation

ABSTRACT

The involvement of genetic engineering techniques in the development of novel biomaterials has a huge impact on a vast range of applications. The capability of new genetically engineered material has achieved various innovative scopes in the biomedical industry. Such materials are usually designed via chemical and physical methods of genetic engineering. According to the genetic basis of sequence, molecular weight, folded structure, and stereochemistry, protein polymers thus suggest a generous view for the architecture of protein-based genetically engineered biomaterials.

The scopes of developing genetically engineered biomaterials are leading to improve biological features of materials which can enhance the applicability and properties of materials. In the last five years, Genetic engineering research is becoming closer to the mass consumer. Leading global geneticists predict that in the coming years, a boom will occur in the genetic engineering market, comparable to the massive spread of personal computers in the 1980s. Thus genetically modified biomaterials with upgraded biological properties, expanding towards mass-scale industrial production, and the considerable consumption in regular universal activities.

The techniques used to develop new materials and to modify the properties of existing materials, are subjected to different industries and fields of scientific researches. CRISPR is an authoritative research tool that facilitates scientists to deal with the expression of a gene. It has shown tremendous potential in genome research due to its ability to delete unwanted traits, and possibly even replace them with desirable traits. It is agile, worthwhile, and more authentic than any preceding gene-editing techniques. Genetically engineered biomaterials have been an enormous field of research over the last fifteen years and CRISPR has already initiated performing a significant aspect in boosting biomaterial research.

Keywords: Genetic engineering; CRISPR; Biotechnology

INTRODUCTION

People have applied biotechnology operations, such as selectively breeding animals and fermentation, for thousands of years [1,2]. Late 19th and early 20th century explorations revealed how microorganisms accomplish commercially advantageous procedures and how they provoke disease contribute to the industrial production of vaccines and antibiotics [3,4]. Upgraded approaches for animal breeding have also emanated from these ventures [5]. Scientists within the San Francisco Bay Area took a large leap forward with the invention and development of recombinant DNA techniques in the 1970s [6-9]. The area of biotechnology proceeds to expedite with modern revelations and unique applications predicted to aid the economy throughout the 21st century [10-12].

Gene targeting is a particular technique that uses homologous recombination to shift an endogenous gene and can be used to eliminate a gene, omit exons, insert a gene, or include point mutations [13]. Genetic engineering has applications in medicine, research, industry, and agriculture and can be used on different types of plants, animals, and microorganisms [9,14].

Genetic engineering has staged a collection of drugs and hormones for medical use. One of its initial applications in

Correspondence to: Withanage SR, ITMO University, SCAMT Laboratory, Saint Petersburg, 191002, Russian Federation, E-mail: withanage@scamt-itmo.ru

Citation: Withanage SR, Daniil K (2020) Genetically Engineered Biomaterials. Adv Genet Eng 9:161. doi: 10.35248/2169-0111.2020.9.161

Received date: May 07, 2020; **Accepted date:** May 15, 2020; **Published date:** May 25, 2020

pharmaceuticals was gene splicing to synthesize large amounts of insulin, made using cells of *E. coli* bacteria [15]. Interferon, which is applied to eradicate specified viruses and kill cancer cells, also is an outcome of genetic engineering, since the tissue plasminogen activator and urokinase, which are used to crumble blood clots [16].

Biomaterials are materials with the origin of nature, either from plants or animals, and have fascinated material scientists for many years due to the consternate-influential properties such as strength, toughness, and adaptability [17]. Some examples of biomaterials are silk, collagen, cellulose, and starch [18]. These constituents of nature have motivated synthetic biologists to genetically engineer organisms to boost upon and develop materials with exceptional properties [19].

Genetically engineered materials such as leather, synthetic silk, and hydrogels are biodegradable, eco-friendly, and can be feasibly manufactured, formulating them an outstanding substitute to their natural variants [20-22]. Biomanufacturing is a type of manufacturing technology that composes biological systems to develop commercially-relevant biomaterials and biomolecules for applications in medicine, food, and other industrial applications. Genetically-engineered materials that simulate the naturally-derived ones are rapidly-growing industry recently [20].

There are several types of development of genetically engineered biomaterials, but the main mechanism and the idea show relativity among each other [2,6,23]. The procedure of cloning of genes in relevant to produce genetically engineered biomaterials, is the main and initiating step. There are different types of cloning vectors are using in gene cloning such as plasmids, bacteriophages, cosmids, yeast cloning vectors, Ti and Ri plasmids, and so on [24].

The selected characteristic of a material is carried out by its responsible gene which is located in the genome of its origin [8]. Using genomic editing methods such as CRISPR/Cas9, the genes are inserted or deleted in a genome compatible with its effect [13]. The selected section of the genome, fix with the plasmids usually using restriction enzymes and ligases. Designed plasmids inserted to the host cell leading to regulation and multiplication of gene as well as to produce compatible target proteins [25].

Genetically engineered material researches are involved in a variety of applications such as drug delivery, nanoparticle coverings, macromolecular carriers, hydrogels; tissue engineering has a huge impact on new innovative methods regarding gene engineering. Researches confirmed different methods of obtaining, developing, and applying these materials with illuminating their developed functions and properties [2,13,21-23] (Figure 1).

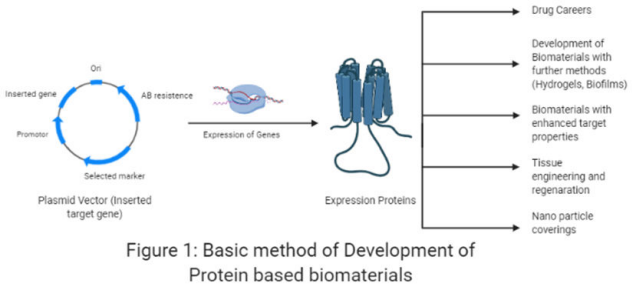

Figure 1: Basic method of Development of Protein based biomaterials

Figure 1: Basic method of development of protein based biomaterials.

GENETICALLY ENGINEERED BIOMATERIALS

Second generation genetic engineering biomaterials

The second-generation genetic engineering biomaterial production technology developed by Chinese scientists in the United States uses microalgae gene technology to produce expensive genetic technology biological products at low cost. The first product of this technology is SRT-type spider silk material, which fills the gap of traditional genetic engineering. It has super strength and toughness. It is a protein matrix with spider silk and has thermoplasticity. Its performance exceeds that of spider silk in the wet form [24,25]. It is used in bulletproof vests, parachutes, airbags, bone regeneration brackets, 3D printing, and high-end fabrics. DuPont already sells similar materials Kevlar: $ 1.5 billion in annual revenue. The performance of this material is close to that of spider silk, surpassing Kevlar, and there is a by-product bio-oil, which will be sold at the price of green crude oil, which can be supported by policies. This technology can maintain similar production efficiency in the laboratory when scaling up, and its reactor can be scaled up by simple replication. At the same time, this technology has environmental advantages. Based on photosynthetic production, it can absorb carbon emissions and produce by-product bio-oil. The project will also develop other genetic products suitable for the production process, such as phycoerythrin, animal vaccines, and seedling growth factors [26].

Natural and genetically engineered proteins for tissue engineering

In 2012, Sílvia Gomes, Isabel B Leonor, João F Mano, Rui L Reis, and David L Kaplan from a collaboration of University of Minho, Portugal, Institute for Biotechnology and Bioengineering, Portugal and Tufts University, Medford, Massachusetts, USA have established a technology to observe properties and applicability of chimeric protein-based biomaterials synthesized through recombinant DNA technology. The recombinant proteins were designed with the cDNA of natural origin (Silk, Chitosan etc.) combining them with the cDNA with bioactive proteins (TGF, EGF etc.). Genetically engineered materials (Proteins) with a combination of natural and biological domains consisted of a variety of chemical domains that allows fusion proteins, cell adhesion, and

increased mechanical properties, anti-microbial ability, and so on. The applications of these materials are likely suggested to be involved in Tissue replacements, Drug delivery, gene delivery, cell encapsulation, and coatings [6] (Figure 2).

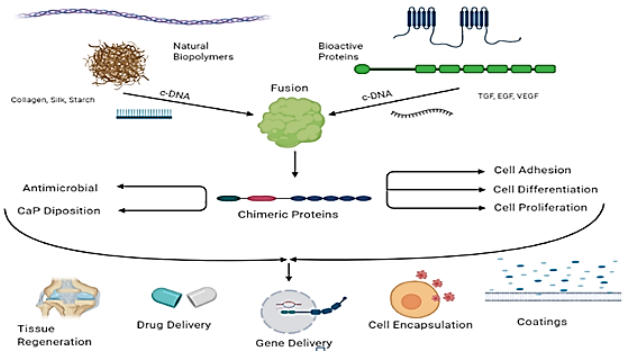

Figure 2: Fusion of c-DNA of natural and bioactive polymers.

Genetically engineered elastin based bio materials

In recent research history of genetically engineered biomaterials, Mercedes Santos, Sofía Serrano-Dúcar, Juan González-Valdivieso, Reinaldo Vallejo, Alessandra Girotti, Purificación Cuadrado and Francisco Javier Arias in University of Valladolid, Spain, have designed genetically engineered Elastin like Recombinants (ELRs) leading to apply them in variety of biomedical applications. Elastin like polypeptides found in nature, consists of short pentapeptide polymers usually the sequence of VPGXG, where X is the variant. X can be any amino acid except Proline. ELRs produced using these ELPs biosequences. ELRs designed using genetic engineering with controlled production over amino acid sequences. This genetically engineered sequence allows different functionalities of ELRs which can be possibly applied in vast range of applications [26,27].

In drug delivery studies, different uses of ELRs were observed in different processes of drug delivery such as soluble drug delivery patterns when combined with therapeutic agents, Pharmacokinetic enhancers, fusion protein expression when combined with drugs and chemical conjugation [2,28]. The modified and existing chains of ELRs consist of different chemical functional groups which allow therapeutic agents to make covalent bonds and express therapeutic effects in damaged tissues. ELRs allow systemic delivery and local delivery of therapeutic drugs, with the preparation of self-assembly in response to external stimuli. ELRs that are soluble at body temperature has been observed as a result of local overheating when accumulate in tumor tissues after thermoresponsive transition. The local delivery of ELR conjugates into tumor tissues can be enhanced by therapeutic hyperthermia by taking advantage of tumor vasculature permeability and perfusion [29,30].

Besides, the ELR based nanoparticle formation and conjugation with drugs and therapeutics to form drug nanocarriers were observed using different strategies such as metal ion-induced self-assembly which allows polypeptide assembly and interchain crosslinking. The applications of these ELR nanoparticles with surface growth factors were evaluated in applications of various biomedical aspects such as chronic skin wound healing, induction of bone regeneration, and treatment of neuronal injuries. Other nanoparticles of ELRs suggested to be applying in various therapeutic methods which can combine with therapeutic agents and these particles can initialize therapeutic agents, drugs, and DNA as well. These effects are suggested to be applied in gene therapy [2,29].

ELR based macromolecular structures such as depots, hydrogels and macromolecular carriers have potential applicability to involve in anticancer therapy, since these structures have ability to respond to the changes such as temperature [31]. Fused ELR based depots can be involve in treatments for agents. ELR based hydrogels are suggested to be apply in sustained release of drugs, growth factors, antibodies and hormones due to their ability of prolonged delivery and minimized effects [32]. ELR based macromolecular hydrogels have potential to be used in tissue engineering such as skeletal system and vascular soft tissues engineering. Bi-component with ELRs such as ELR-collagen, ELR-silicon has potential effects of involving in tissue engineering [2] (Figure 3).

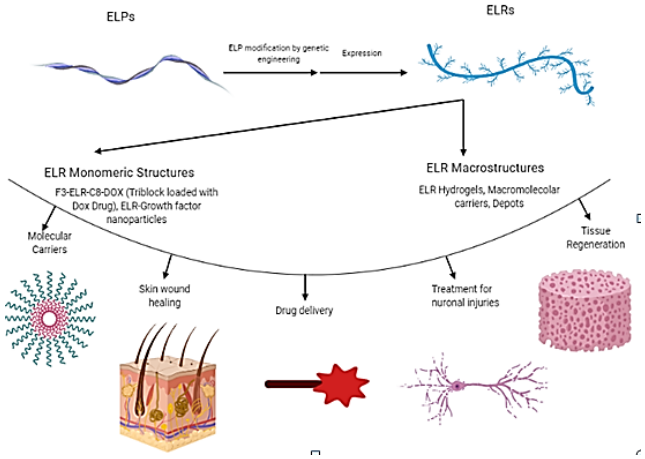

Figure 3: ELR based biomaterials classification and suggested applications.

"Genetically engineered" bio functional triboelectricnano generators using recombinant spider silk

Yujia Zhang, Zhitao Zhou, Long Sun, Zhen Liu, Xiaoxia Xia, and Tiger H. Tao conducted a project to genetically engineer spider silk and obtained the expression proteins leading to apply in a variety of biomedical applications. The recombinant plasmids were produced dragline like silk consist of different repetitive units [22].The silk expression plasmids were contained in *E. coli* cells and the expressed proteins were obtained after a series of chemical treatments and purifications. In Figure 4, the extraction and purification procedure with chemical treatments are indicated. The genetically engineered silk proteins were obtained and their characteristics were obtained. Hence genetically engineered Spider silk material has used as modern triboelectric biomaterial. Triboelectric materials are such materials which can generate electron flow by collision leading to generate electricity [33,34]. The newly developed material is suggested to acquire biocompatible, multifunctional, consist of

programmable triboelectric properties, the capability to fabricate in large scale, and transcendent output performance. These excellent properties of the genetically engineered spider silk proteins enhance the range of the aspects of researches, relevant to silk-based materials. Besides, the large scale production of this material is environmental friendly [32] (Figure 4).

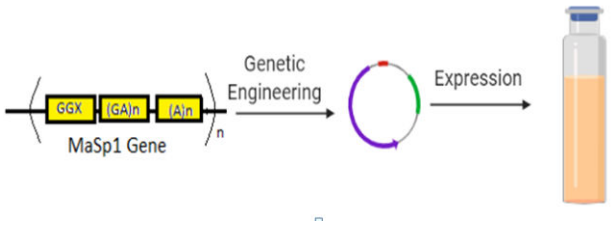

Figure 4: Expression of genetically modified spider silk material.

As well as, within this mass manufacturing, scientists have introduces water lithography techniques to Recombinant Spider Silk Proteins and Triboelectric Nanogenerators along with easily adjustable surface morphological surfaces, surface properties with possible chemical modification capability and to attain controllable protein conformational changes and structural properties. The recombinant spider silk proteins were mixed with Graphene (GR), Carbon Nanotube (CNT), and drug molecules through a simple mixing process [35].

The functionalized solutions transferred on to the Polyethylene Terephthalate (PET)/ Indium Tin Oxide (ITO) substrates and enhanced the triboelectricity by water lithography which produced rough surface patterns on RSSP and allows integrating functional molecules and patterns on TENGs.

RSSP-WL-TENGs were prepared followed by water/ethanol development for 12 hours and assembling with another arch-shaped PET/ITO. The triboelectric capabilities and the generated electricity have been increased by increasing the repetitive (MaSp1) units in the RSSP chains. As well as, in this experiment, the scientists have suggested the self-powered applications of recombinant Spider silk protein-based materials [36]. The voltages through the RSSP-WL-TENGs were compared in general patterns with multiple layers printed patterns. They have observed low voltage when the distance between the layers are increasing and with increasing of the number of printed layers, the voltage has been increased up to 4th layer shows the maximum voltage and after the 4th layer, the voltage has been gradually and slightly decreased compared to the voltage measures after 4th layer [37]. And also, the output voltage has observed and compared with and without surface modification of RSSP with graphene, carbon nanotubes, Au, and Ag. With each of the modified components with RSSP showed several times higher output voltage than its output voltages. Thanks to these excellent triboelectric properties, they have lighten1880V and 2600V commercial LED by hand patting RSSP-WL-TENGs. While this process, they have experienced that there are electrical sparks indicated when the high output voltage reaches to the electrical breakdown threshold of air [38]. They have compared this triboelectric properties of the RSSP-WL-TENG materials with previously designed triboelectric materials such as Cellulose/FEP, Gelatin/PLA, Silk/PI, Silk/PET and PDMS/Gold [39] They have found that this material consist of extraordinary triboelectrical properties compared to those materials [40].

Besides, in relevant to this project they have observed the antimicrobial ability of genetically engineered recombinant spider silk proteins combined with different materials doping with metallic nanoparticles, GR, and CNT. They have observed the antibacterial ability of different conjugates with RSSP, such as RSSP alone, RSSP/GR, RSSP/Ag, and RSSP/GR/Ag with and without triboelectrical activation. They have observed excellent anti-microbial abilities with gram-positive and gram-negative bacteria and the combination of RSSP/GR shows the highest antimicrobial ability without triboelectric excitation. With triboelectric excitation, the RSSP/GR/Ag showed the highest antimicrobial ability. By the way all the combination materials used combined with RSSP indicates optimum antimicrobial abilities over gram negative and gram positive bacteria leading to apply them in antibacterial materials such as drug-free antimicrobial patches [41]. Finally they have designed anti-microbial patches with RSSP conjugated TENGs leading to apply in drug-free antimicrobial products. Hence the genetically engineered recombinant spider silk proteins haspromisive antibacterial effects *in vitro* and *in vivo* systems [42] (Figure 5).

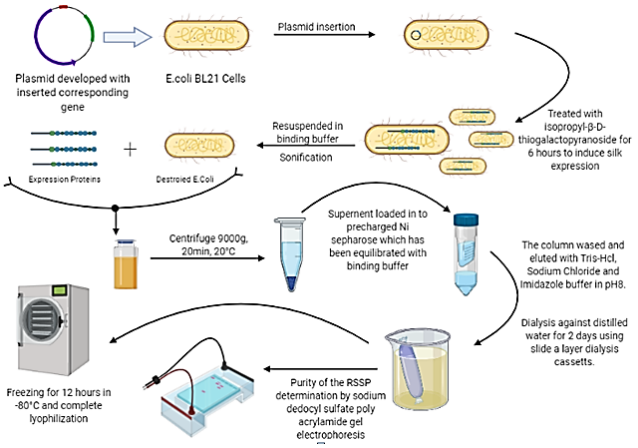

Figure 5: Extraction and purification procedure of genetically engineered RSSP.

Smart and genetically engineered biomaterials and drug delivery systems

Jindrich Kopecek from the Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah has developed this project to implement drug delivery systems with smart and genetically engineered biomaterials [1,23,28,31]. In these experiments they have designed several preparation procedures leading to obtain effective biomaterials in a variety of applications. The applications of these materials are directly aimed on drug delivery [43].

In relevant to stimuli response biomaterials they have discussed mainly about linear polymers, copolymers and hydrogels. The preparation, characteristics, potential applicability, and prospects are also described regarding these materials. Besides, this project mainly aimed at the discussion of genetically engineered protein-based biomaterial production, preparation, and analyze the applicability and further potential perspectives

relevant to make use in biomedical industry [44]. The designing of protein was suggested to express excellent properties leading to improve physical, chemical, and biological characteristics. In this aspect the newly designed proteins are showing extraordinary potentials in a wide range of industrial applications and to reduce worse effects with the materials which had been used instead of these newly designed materials [45]. In the beginning, the protein structures of different materials have been observed and designed specific and characterizing target proteins. According to the amino acid sequence of protein macromolecule they have predesigned, the corresponding genetic sequence was discovered. Then the corresponding oligonucleotide sequence for the designed protein was chemically synthesized. In this case, the longer or more complicated chains were prepared based on stepwise ligation methods. The whole sequence was divided into several parts and chemically synthesized before ligating the separately prepared whole sequence. PCR technology was used to amplify the chemically designed nucleotide chains and the designed sequence ligated with plasmid vectors. The plasmid vectors express proteins relevant to the nucleotide sequence and which can be accumulated in the cytoplasm of bacteria as a soluble material, inclusion bodies as insoluble form, and either secretion out of the periplasmic area through the cell membrane. In this case, they have mentioned Immobilized Metal Affinity Chromatography (IMAC) is the most convenient technology to purify the processed proteins [46,47] (Figure 6).

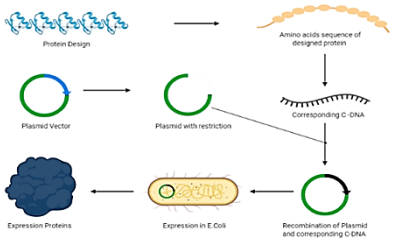

Figure 6: Designing and expression of natural proteins.

In relevant to the biomedical applications of genetically engineered materials mainly drug delivery systems were discussed in this project with considering different relevant results of previous research works. In 1980, the first attempt to design genetically engineered polymer was reported and clones which consist of 150 repetitive units of Aspartyl-phenylalanine were produced [48]. This materials was used to produce artificial sweeteners Asp-Phe. In addition to that, the incorporation of non-natural amino acids leading to produce genetically engineered biomaterials was discussed considering different explanations. A modern approach for incorporation of unnatural amino acids is to modify the specificity of aminoacyl-tRNA synthetases by site directed mutations [49]. This technique can facilitate the non-natural amino acids to bind in protein chains produced in *E. coli*.

Besides that, the Hybrid hydrogels containing protein domains, Associative oligopeptides and block copolymers, Pharmaceutics related applications of smart materials were discussed. In the main part, genetically engineered scaffolds for display of receptor binding epitopes, drug delivery mediated by protein polymers, smart hydrogels have potentials in drug delivery were deliberated [23] (Figure 7).

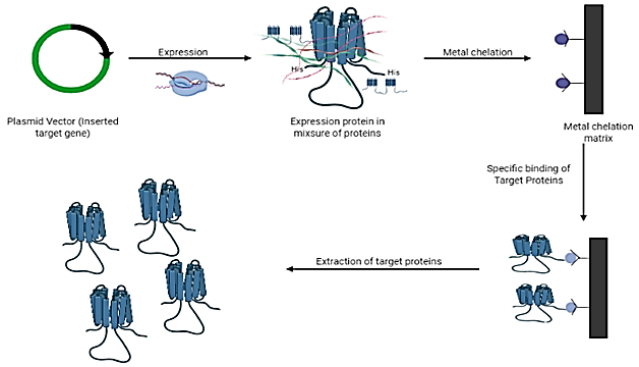

Figure 7: Purification of targeted expression proteins by metal affinity chromatography.

Thus, novel materials of unique properties were designed and evaluated. Currently, researchers are persuading different techniques to redesign and to develop natural biomaterials. The main aims of the current researches regarding the development of biomaterials using genetically engineering techniques are mainly focused on biomedical and medical material industries. Besides the main scopes, number of researches and current projects are developed to preparation of genetically engineered biomaterials leading to provide solutions for timely global problems.

Novel genetically engineered human osteoblasts for the *in vitro* study of biomaterials

Researchers in University of Oulu performed this project to develop human osteoblasts using genetic engineering procedure. Tissue engineering has been fast growing fields of biomedical sciences and technologies. The ultimate scope of the tissue engineering and biomaterials are the *in vitro* and *in vivo* regeneration and development of functionally active tissues. In this experiment, a cellular model relevant for the *in vitro* interpretation of cell characteristics, arrangements and proliferation on biomaterials was observed. Human osteosarcoma derived cell line which named Saos-2 was genetically modified and this Saos-2 presented the osteoblastic phenotype [40-42], and maintained an absolute proliferation possibility *in vitro*. pCMV-eGFP expression vector was used to transfect daughter Saos-2 cells. The pCMV-eGFP vectors were holding enhanced green fluorescent proteins as a reporter gene. The genetically homogenous immortal cells named Saos-eGFP was resulted and proliferated in culture followed by Genetic in selection. Saos-eGFP cells showed the morphological and cytological features of osteoblast cells and it expressed the fluorescent properties of eGFP. The intensified form of this Green Fluorescent Protein was produced by mutation of gene extracted from the jellyfish Aequorea victoria. This GFP showed fluorescence properties without producing toxic effects. The physiological expression of the bone tissues were not affected or deteriorated by GFP presences and the comparative observations with parent cells with engineered cells presented that the expression of both of these types produce bone tissue specific molecular markers such as osteocalcin and osteopontin. Besides,

the observations suggested that there were no functional modifications and functional differences of both parent and modified cells (Figure 8).

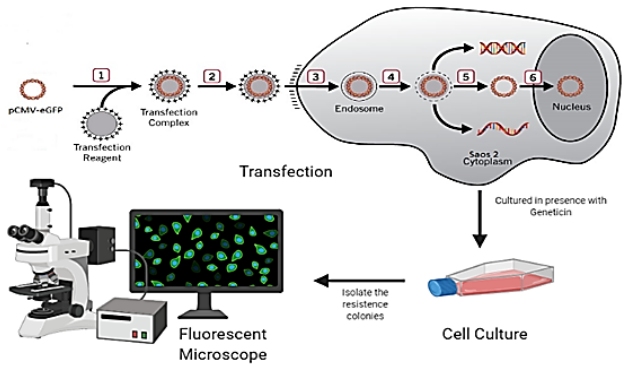

Figure 8: Transfection of pCMV-eGFP in to Saos- 2 cells, culture and fluorescent microscopy.

Parental and genetically engineered cells were interrogated by immunofluorescence assay to observe the cytoskeletal architecture. The main proteins of cell architecture, actin fibers were not affected by exogenous proteins and identified compatible cellular architectures between parental and engineered cells. The fluorescence intensity measurements in cells were measured and found that the fluorescence intensity is directly proportional to the presence of GFP genes and proteins. The GFP was proportional to the number of cells. In this aspect, the intensity of fluorescence was taken as the measurement to evaluate the number of cells [44].

The Saos-eGFP cells produced in this study suggest a compatible *in vitro* structure for the visualization of the mature osteoblastic cell morphology and proliferation on biomaterials describing certain and specific surface, topographical or chemical characteristics. In addition, variety materials with certain morphology, architecture and configuration may interfere with the proliferation and the propagation pattern of osteoblasts on material surface. Mature osteoblasts on the Carbon Fiber Reinforced Polymers (CFRP) surface showed increased homogeneous propagation and enhanced rate of proliferation than those on the Polyether Ether Ketone (PEEK) polymer surface under equal culture conditions. This observation describe that the chemical characteristics, the architecture of the surface and the visualization of the CFRP crates attain as substantially various effects on the mature osteobalsts than PEEK cages.

Incorporating the BMP-2 peptide in genetically-engineered biomaterials accelerates osteogenic differentiation

This project was developed to incorporate the (Bone Morphogenic Protein) BMP-2 peptide leading to apply in tissue engineering and it scaffolds as backbone of biomaterials based on proteins leading to osteogenic differentiation of mesenchymal cells. BMP-2 is suggested to be effective growth factor on mesenchymal stem cell to differentiate in to osteogenic cell leading to generation of bone tissues. The BMP-2 is in a soluble form which can be affected with several factors in production, purification and extraction. The production of recombinant (rhBMP-2) is expensive and the production of rhBMP-2 in mammalian cells are very low while the production in bacterial cells express rhBMP-2 in inclusion bodies which is complicated to purify. Besides the efficient clinical concentrations of BMP-2 produces adverse effects on human such as inflammations and swelling. The retention time and half-life of rhBMP-2 suggested being very short [45].

In this case, as an alternative for BMP-2 peptide, short fragment of BMP-2 incorporated with scaffold material which was designed and genetically engineered in this project. The analysis of the amino acid sequence was done incorporating multiple functionalities to the designed protein backbone leading to promote the osteogenic effects, adhesion effects and hMSC proliferation activity. In the preparation process of this novel genetically engineered biomaterial, resin like protein and BMP (RZ-BMP) was prepared and showed that the hMSC proliferation on the surface and hMSCs excellent levels of AP activity, calcium deposition and gene expression of Runx2 Type 1 collagen. As well as the RZ-BMP proteins were highly likely to be specific on osteogenesis, since the observations proved that there is no increased expression of cartilage markers such as Collagen type 2. The bio activity of the RZ-BPM was not depending on the protein desorption from the medium. Thus, the material confirms the bioactivity within expected context and highly specific on osteogenesis. They have also taken an approach to observe the similar effects with the preparation of RZ-scRGD (RGD cell binding motif), RZ-RGD and RZ-scBMP. RZ-scBMP did not show expectable levels of calcium deposition and did not show enhanced osteogenic markers as RZ-BMP did. RZ-BMP+RZ-scRGD showed osteogenic differentiation like RZ-BMP performed alone. And they have mentioned that the AP activity and calcium deposition are equivalent among these two groups. Up regulation of more osteogenic genes were performed with RZ-BMP+RZ-scRGD than RZ-BMP. RZ-BMP+RZscRGD expressed Runx2 in seven days, AP in 4 days and type 1 collagen in 4 days. By the way, RZ-BMP and RZ-BMP+RZ-scRGD both performed excellent osteogenic differentiation [45] (Figure 9).

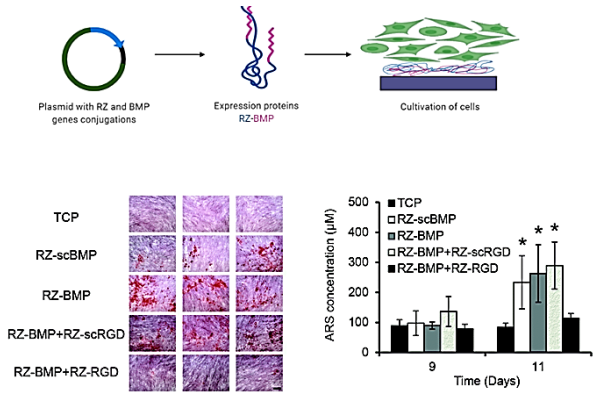

Figure 9: Expression and cultivation of HMSC, differentiation of cells and results of Alizarin red staining quantification assay to observe osteogenic differentiation.

Besides they have found that the BMP-2 protein concentration on RZ-BMP+RZ-scRGD is lower than that of BMP-2 concentration on the RZ-BMP. The RZ-BMP showed almost

twice BMP-2 peptide density than RZ-BMP+RZ-scRGD surfaces. Comparing the results, the osteogenesis occurred and the bio active ligand BMP-2 did not showed such significant effects on osteogenesis with in the peptide density range of 33 pmol/cm^2-60 pmol/cm^2. The RZ-BMP+RZ-RGD did not perform considerable effects on osteogenesity. But it showed effects of osteogenic gene expression. Hence, the project suggested optimum and extraordinary aspects of incorporation of bioactive ligand BMP-2 with proteins leading to promote osteogenic differentiation towards the development of tissue engineering.

'Bacterial builders' produce functional biomaterials

Wyss Institute researchers have developed a modern, adequate and measurable technique for construct biomaterials using *E. coli* bacterial colonies as biofactories from protein structures called 'amyloids'. Amyloids are fibrous structures consist of nanoscale features, and accompanied by a range of biological behaviors. These are occurring naturally in a normal environment. They were exactly suggested to be an indication of Alzheimer's and Parkinson's diseases caused by protein miss-folding. "Functional amyloids" were found to be attaining advantageous aspects in the preservation of organisms and their connections with surfaces, including insects and bacteria [46].

There is a wide range of applications of amyloid biomaterials such as tissue engineering, water purification, vaccine production and so on. But still, there is no such implemented way to proceed with the amyloid protein production in large scale. Existing methods to the production of amyloid proteins are depending on extracted amyloids from natural sources. Besides, it was hard to differentiate amyloid leading to the applications and the purification was also an expensive and time-consuming procedure after recombinant amyloid production in *E. coli*.

Neel Joshi, Ph.D., has designed a modernized, vacuum filtration technique that allows agile separation of amyloid fibers from *E. coli* cultures after genetic engineering of amyloid protein fibers using *E. coli* as an expression host. This method depends on the probability of a certain grade of amyloid fibers to persist unharmed even in the existence of hazardous detergents and certain enzymes that will break down cells, DNA and other proteins. Besides, it doesn't require any specially designed equipment and sidesteps leading to acquire slow purification steps. This developmental technology provides an extraordinary interpretation of current approaches to protein-based materials fabrication and also simplifies and enhances protein crops compared to previous techniques. This technique allows the extraction and purification of genetically engineered amyloids more specifically in different grades in relevant to a variety of applications.

Gene activated adipose tissue fragments as advanced autologous biomaterials for bone regeneration:

Osteogenic differentiation within the tissue and implications for clinical translation

Bin Ren, Volker M. Betz, Roland M. Klar, Volkmar Jansson, Peter E. Müller and Oliver B. Betz from Department of Orthopedic Surgery, Physical Medicine and Rehabilitation, University Hospital Grosshadern, Ludwig-Maximilians-University Munich, Germany have developed a cost-effective method for osteogenesis. As mentioned in topic 1.7, BMPs are effective bioactive proteins for osteogenesis and differentiation. By the way, the half-life of BMP is short and to overcome this problem, they have developed genetically modified cells. Since the problematic conditions when the isolation and cultivation of cells and while transferring to clinics, the scientists developed gene activated fragments of adipose tissues which has great bone repair effects. Besides they have presented that adenoviral transduction with human BMP-2 can facilitate osteogenic differentiation within adipose tissue segments, once present in an in-vitro culture system. They have used quantitative reverse transcriptase-polymerase chain reaction, immunohistology and histomorphometry to evaluate the osteogenesis induction [47].

BMP-2 transduced adipose tissue was prepared and observed over 30 days for osteogenic differentiation. Osteogenic differentiation was induced with the calcium deposition, up-regulation of bone markers and expression of osteogenic proteins. Mainly this project presents that cells within adipose tissue segments can differentiate osteogenically followed by BMP-2 transduction of cells on the surface of the adipose tissue. BMP-2 gene activated adipose tissue provide excellent capabilities of differentiation into bone tissue [47,48].

FURTHER RESEARCHES

Further researches relevant to genetically engineered biomaterials are slightly improving and some of them are listed below.

- Engineering a living biomaterial via bacterial surface capture of environmental molecules [49]
- Scalable production of genetically engineered nanofibrous macroscopic materials via filtration [50]
- Electrospun silk-elastin-like fibre mats for tissue engineering applications [51]

DISCUSSION AND CONCLUSION

In relevant to the genetic engineering towards the improvement of biomaterials leading to different applications are increasingly boosting up research scope as we have discussed in the introduction. We have discussed and described several important projects which were preceded by leading researchers and research institutions. All the projects were analyzed and evaluated in relevant to their impact, importance and necessity.

Different techniques of genetic engineering have been used leading to the development of novel biomaterials and to improve and enhance the properties of existing materials. Plasmid gene delivery, genetic transformation, genetic transduction, gene transfection using different types of expression plasmids and viral vectors or cosmids was observed

leading to genetically modify the materials. There are a wide range of genetic modifications have been done according to the recent research references. The develop biomedical properties as well as improving chemical and physical properties leading to expand the applicable capacity are highlighted remarks along with the development of biomaterials with genetic engineering technologies. Genetically engineered biomaterials were suggested to have a huge impact on biomedical applications such as drug delivery, tissue engineering, vaccine production, biological visualizations and so on. Physical and chemical characteristics improved genetically engineered biomaterials allow them to be involved in water purification, using as Nano-generators and more. There is another type of secondary applicability of genetically engineered biomaterials. The use of genetically engineered proteins in the production of hydrogels, films, matrices, pharmaceutical materials and different types of physiological and anatomical implants can be able to provide extraordinary materials with excellent multiple properties leading to widen the range of applicability.

FUTURE PROSPECTS

In terms of the genetically engineered materials, the research scope is still in the level of infantry stage since the field has great potentialities to be applied in relevant to provide exceptional solutions for global problems as well as biological, medical, physical and chemical applications. Genetically engineered proteins can be able to develop novel biomaterials with biomedical applications as discussed. These techniques can be developed in the near future with such properties of biocompatibility, biodegradability, potential energy harvesting capabilities, thermal properties, optical properties, and carcinogenic detection potentialities and so on. Tissue engineering for bone tissue regeneration and differentiation methods have already developed and the applications of these genetically engineered materials will be available in industrial clinical settings in the near future. As an example for future developments of these methods, scientists from the University of Michigan are currently working on a project to develop genetically engineered new biomaterial to detect the early cancer diagnostic method instead of currently using invasive clinical diagnostic methods such as tissue biopsies. According to NCBI, these are a huge number of professors discussing and working on leading to develop characteristics and properties of biomaterials. In relevant to these discussions, genetic engineering techniques will be playing a major role in the development of future biomaterials. To satisfy human and global needs, genetic engineering will provide exceptional outcomes in the future by facilitating accessible, comfortable and conducive functionalities.

REFERENCES

1. Goldberg M, Langer R, Jia X. Nanostructured materials for applications in drug delivery and tissue engineering. J Biomater Sci Polym Ed. 2007;18:241-268.
2. Santos M, Serrano-Dúcar S, Gonzalez-Valdivieso J, Vallejo R, Girotti A, Cuadrado P, et al. Genetically engineered elastin-based biomaterials for biomedical applications. Curr Med Chem. 2018;25.
3. Meyer DE, Chilkoti A. Genetically encoded synthesis of protein-based polymers with precisely specified molecular weight and sequence by recursive directional ligation: examples from the elastin-like polypeptide system. Biomacromolecules. 2002;31:357-367.
4. Jain RK. Delivery of molecular medicine to solid tumors. Sci. 1996;271:1079-1080.
5. Cappello J. The biological production of protein polymers and their use. Trends Biotechnol. 1990;8:309-311.
6. Gomes S, Leonor IB, Mano JF, Reis RL, Kaplan DL. Natural and genetically engineered proteins for tissue engineering. Prog Polym Sci. 2012;37:1-17.
7. Svab Z, Hajdukiewicz P, Maliga P. Stable transformation of plastids in higher plants. Proc Natl Acad Sci USA. 1990;87:8526-8530.
8. Minkenberg B, Wheatley M, Yang Y. CRISPR/Cas9-Enabled multiplex genome editing and its application. 2017;149:111-132.
9. https://www.ncbi.nlm.nih.gov/books/NBK215771/
10. http://www.chiropractic.org/wp-content/uploads/2018/12/1200-studies-The-Truth-Will-Prevail-3.pdf
11. Khan S, Ullah MW, Siddique R, Nabi G, Manan S, Yousaf M, et al. Role of recombinant DNA technology to improve life. Int J Genomics. 2016;2016:2405954.
12. Velazquez-Campoy A. Biotechnology in the 21st century: Challenges and opportunities. Anales de la Real Academia Nacional de Medicina. 2018;135:169-173.
13. Gerlai R. Gene targeting using homologous recombination in embryonic stem cells: The future for behavior genetics? Front Genet. 2016;7:43.
14. https://www.ncbi.nlm.nih.gov/books/NBK217989/.
15. Baeshen NA, Baeshen MN, Sheikh A, Bora RS, Ahmed MMM, Ramadan HAI, et al. Cell factories for insulin production. Microb Cell Fact. 2014;13:141.
16. Takaoka A, Hayakawa S, Yanai H, Stoiber D, Negishi H, Kikuchi H, et al. Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence. Nature. 2003;424:516-523.
17. Williams DF. Definitions in biomaterials, proceedings of a consensus conference of the European society for biomaterials. Amsterdam:Elsevier, 1987.
18. Yadav P, Yadav H, Shah VG, Shah G, Dhaka G. Biomedical biopolymers, their origin and evolution in biomedical sciences: Asystematic review. J Clin Diagn Res. 2015;9:ZE21-ZE25.
19. Shapira P, Kwon S, Youtie J. Tracking the emergence of synthetic biology. Scientometrics. 2017;112:1439-1469.
20. Zhang YP, Sun J, Ma Y. Biomanufacturing: history and perspective. J Ind Microbiol Biotechnol. 2017;44:773-784.
21. Zhang L, Xiang Z, Zhao G, Wu Z, Cui H. Functionalized genetic engineered silk-based biomaterials and their applications. Chinese J Biotechnol. 2019;35:956-971.
22. Zhang Y, Zhou Z, Sun L, Liu Z, Xia XX, Tao T. "Genetically engineered" biofunctional triboelectric nanogenerators using recombinant spider silk. Advanced Materials. 2018;30:1805722.
23. Kopeček J. Smart and genetically engineered biomaterials and drug delivery systems. Eur J Pharm Sci.2003;20:1-16.
24. Khan K. Vectors used in gene manipulation-a retrospective. Advanced Biotech J. 2009:1-8.
25. https://www.ncbi.nlm.nih.gov/books/NBK21498/.
26. https://ejournal.stpi.narl.org.tw/sd/download?source=1070310.pdf&vlId=82be405304c046a2a14713153bf6f778&nd=1&ds=1

27. Xiaowen L, Wang C, Liu Z. Protein-engineered biomaterials for cancer theranostics. Advanced Healthcare Materials. 2018;7:1800913.
28. Lattin JR, Belnap DM, Pitt WG. Formation of eliposomes as a drug delivery vehicle. Colloids Surf B Biointerfaces. 2012;89:93-100.
29. Sarikaya M, Tamerler C, Jen AK-Y, Schulten K, Baneyx F. Molecular biomimetics: Nanotechnology through biology. Nat Mater. 2003;2:577-585.
30. Langer R, Tirrell DA. Designing materials for biology and medicine. Nature. 2004;428:487-492.
31. Agrahari V, Mitra AK. Nanocarrier fabrication and macromolecule drug delivery: Challenges and opportunities. Ther Deliv. 2016;7:257-278.
32. Zou H, Zhang Y,Guo L, Peihong W, He Xu. Quantifying the triboelectric series. Nat Comm. 2019;10:1427.
33. Ferrari FA, Cappello J. Biosynthesis of protein polymers. Protein-Based Materials. 1997:37-60.
34. Handehari G, Capello J. Genetic engineering of protein-based polymers: Potential in controlled drug delivery. Pharm Res. 1998;15813-15815.
35. Bittolo BS, Rapi M, Coletta R, Morabito A, Valentini L. Plasticised regenerated silk/gold nanorods hybrids as sealant and bio-piezoelectric materials. Nanomaterials (Basel). 2020;10:179.
36. Chunhua Y, Xin Y, Yanhao Y, Zhiyong C, Xudong W. Chemically functionalized natural cellulose materials for effective triboelectric nanogenerator development. Advanced Functional Materials. 2017;27:1-7.
37. Block H, Maertens B, Spriestersbach A, Brinker N, Kubicek J. Immobilized-metal affinity chromatography (IMAC): A review. Methods Enzymol. 2009;463:439-473.
38. Doel MT, Eaton M, Cook EA, Lewis H, Patel T. The expression in E. coli of synthetic repeating polymeric genes coding for poly (L-aspartyl–L-phenylalanine). Nucleic Acids Res. 1980;8:4575-4592.
39. Tang A, Kopec˘ek J. Presentations of epitopes on peptide scaffolds and selection of lymphoma-targeting moieties based on epitope recognition. Biomacromolecules. 2002;3:421-431.
40. Rodan SB, Imai Y, Thiede MA, Wesolowski G, Thompson D. Characterization of a human osteosarcoma cell line (Saos-2) with osteoblastic properties. Cancer Res. 1987;47:4961-4965.
41. Morelli C, Barbanti-Brodano G, Ciannilli A, Campioni K, Tognon M. Cell morphology, markers, spreading, and proliferation on orthopaedic biomaterials. An innovative cellular model for the "in vitro" study. J Biomed Mater Res A. 2007;83:178-183.
42. MorelliC, Campioni K, Parolin C, Palù G, Tognon M. Activity of the matrix metalloproteinase 9 promoter in human normal and tumor cells. J Cell Physiol. 2004;199:126-133.
43. Opecek J, Kopeckova P, Minko T, Lu ZR . HPMA co-polymer–anticancer drug conjugates: design, activity, and mechanism of action. Eur J Pharm Biopharm. 2000;50:61-81.
44. Tognon M, Morelli C, Ciannilli A, Campioni K, Bona C, Boriani S, et al. A novel genetically engineered human osteoblasts for the in vitro study of biomaterials. Topics in Tissue Engineering. 2008:1-13.
45. Kim Y, Renner J, Liu J. Incorporating the BMP-2 peptide in genetically-engineered biomaterials accelerates osteogenic differentiation. Biomater Sci. 2014;2:1110-1119.
46. McAlpine KJ. Bacterial builders produce functional biomaterials. Wyss Institute. 2016.
47. Ren Bin, Betz Volker, Thirion C, Salomon M, Klar R, Jansson V, et al. Gene activated adipose tissue fragments as advanced autologous biomaterials for bone regeneration: Osteogenic differentiation within the tissue and implications for clinical translation. Sci Rep. 2019;9:224.
48. Poon B, Kha T, Tran S, Dass CR. Bone morphogenetic protein-2 and bone therapy: Successes and pitfalls. J Pharm Pharmacol. 2016;68:139-147.
49. Scott F, Heyde K, Rice MJ, Ruder W. Engineering a living biomaterial via bacterial surface capture of environmental molecules. Synthetic Biology. 2018;3:ysy017.
50. Courchesne NM, Duraj TA, Tay R, Nguyen P, Joshi N. Scalable production of genetically engineered nanofibrous macroscopic materials via filtration. ACS Biomaterials Sci Eng. 2016;3:733-741.
51. Machado R, Costa A, Sencadas V, Garcia AC, Costa C, Padrao J, et al. Electrospun silk-elastin-like fibre mats for tissue engineering applications. Biomed Mater. 2013;8:065009.