Journal of Medical Pharmaceutical And Allied Sciences

www.jmpas.com
ISSN NO.2320-7418

REVIEW ARTICLE

GENE PHARMING

Saifi Zoya, Kulkarni Parag A*

SVKM's, NMIMS,
School of Pharmacy & Technology Management,
Shirpur, Maharashtra. India.

Correspondence
Dr. Parag A. Kulkarni
SVKM's, NMIMS, School of Pharmacy and Technology Management, Shirpur. Maharashtra. India.
parag.kulkarni@nmims.edu

Keywords

Gene Pharming, Genetic Engineering, Transgenic Animal, Surgical Threads, Spider Silk Fibers.

Received
12/07/2018
Reviewed
18/07/2018
Accepted
25/07/2018

ABSTRACT

The term Gene Pharming is a technology to modify the DNA of animals or plants, this modified gene is called as transgene and this transgene is very useful to produce proteins of medicinal value. This article includes new technologies of genetic engineering, which help in producing various proteins for pharmaceutical use. The transgenic animal spider and goat are used to produce a type of protein from spider silk in goat milk, which can replace synthetic fibers. Surgical threads, bandages and even artificial ligament can also be made by the help of these spider silk fibers. Spider goat are the goats which consist of spider genes and they lactate silk of spiders in their milk. Adam Rutherford modified the goat genetically in a farm which is located at the Utah State University and those goats produce ample amount of silk of spider in their milk, it constitute as one of the strongest substances which are known to man.

INTRODUCTION

Pharming- Well by this word, you might be thinking of cyber-attacks but, let me tell you this is not another word which is misspelled. The result of pharming is recombinant proteins. Commonly recombinant DNA is produced in bioreactors by taking the help of bacteria or yeast. But in this technology, production of recombinant DNA's requirement is not an expensive technology and at reduced cost they meet all the demands of large production capacity. The first organism which was genetically modified to produce human proteins is sheep. The proteins are secreted in milk of sheep here; cloning helps to produces much more valuable proteins.[1] Gene Pharming: It is a technology which is used to modify the DNA of animal or plant called as transgene. Thereby this transgene helps to produce proteins of medicinal value. [2] For pharmaceutical proteins it is a well-established system. The gene pharming in plants has already got success in producing different types of technical proteins. The recombinant protein derived from production plants are approaching commercial levels. So, imagining the world where any type of protein could be safely produced in unlimited quantities, inexpensively. Whereas the idea of producing transgenic animals was suggested 20years ago,[3] the milk and blood of transgenic animals are very good source of pharmaceuticals. It is a very slow process but it is in progress to improvise the effectiveness and efficiency of genetic pharming. Some reports estimate that only 10% of recombinant proteins can be produced by the help of transgenic animals. [4] Pharming can also produce individual organs such as heart, liver, and kidney. Therefore the research is going underway into pharming pigs, by producing organs. So that, those organs are going to help and will be used in transplant operation. [5]

WHAT IS THE CHALLENGE

It is difficult to put efforts on producing recombinant spider silk by biotechnological techniques because: Spiders are not able to be farmed like silk worms as per the need; the reason is they come in category of cannibals and so they eat each other. And, the silk produced by spiders are very fine zand also with the help of 400 spiders only 1sq. yard of cloth can be produced. Recently Nexia Biotechnologies Inc. in Montreal Canada inserted silk gene in the goats to get

recombinant spider silk proteins in those goats' milk. They can be also, plants (tobacco), cloned in yeast (for Pichia Pastoris), bacteria (E.coli) insects (for e.g. silkworm larvae). [6] One of the trade mark is Bio Steel for very good quality fiber-based material which is made up of the spider silk protein, removed from the goat's drain, consisting of transgene which was successfully obtained from the Nexia Biotechnologies, and after that it was obtained by the Randy Lewis lab of the University of Wyoming and Utah State University. The spider silk is 8-10 times as stronger as steel if it is compared with the steel of the same weight. It can extend to 20 times to real size of it. It likewise has high protection from extraordinary temperature, without losing any of its properties.[7]

The organization made many goats which helps to deliver recombinant species of the Major ampullatespecie spidroin I or of the dragline I (for its prevalent versatility, adaptability and also quality) by taking the help of Nephila clavipes, the brilliant circle weaver) or Major ampullatespidroin 2 or dragline 2 which is obtained from Nephilaclavipes itself, by producing its proteins in their milk.[8][9] When goats starts lactating out the milk, having the recombinant DNA silk, it is to be gathered and checked by taking the help of chromatographic methods in order to obtain the pure recombinant silk Proteins. The refined proteins of silk have to be dried, broken up utilizing solvents and converted into microfibers by taking the help from wet-turning fiber creation strategies.

The filaments which are stunned are accounted for having scope of 2 - 3 grams/denier and stretching scope of 26-44%. The "biopolymers of Bio Steel" will be changed into nanofibers and also in nanometers by the electrospinnin technique. [10] Nexia depicts as main organization which helps effectively to deliver strands from silk of spider into the goat's milk. The Lewis lab delivered filaments from transgene creepy crawly protein of silk and engineered silk proteins and hereditary delusions created in two of them recombinant E. coli and the drain of goats consisting recombinant silk, be that as it may, nobody has possessed the capacity to create the silk in business amounts up to this point.

The organization was established in 1993. Its founder was Paul Ballard and Dr. Jeffrey Turner, and after that in the year of 2005it

got sold to the Pharmathene. Two trans genetically goats were created and sold, which was took by Canada Agriculture Museum in 2009 after the bankrupt of Nexia Biotechnologies.[11]

Till now research is preceded with assistance of Randy Lewis, a teacher some time ago, at the University of Wyoming and now it is at Utah State University. There are currently around 30 insect goats at a college run farm.[10]

EXPLANATION

Spider silk have extraordinary mechanical properties. Its fibers are made up of a protein based material produced under mid conditions. This article will give uses of silk based materials in the field of biomedicine.[12] In 1980s, by taking the help of recombinant bacteria, human insulin was obtained by the help of bacteria E.coli, which is now used by all patients having diabetes.[13] Dragline silk is the most important variety of spider's silk; their mechanical properties have been analyzed whereas Madagascar has been discovered. They spin single thread with the length of about 25cm. The University of California, Santa Barbara team used AFM i.e. Atomic Force Microscopy. They analyzed protein solution which was synthesized from dragline silk. They observed that protein solution is assembled in nanofibers in a stack pattern producing a Nano crystal with amorphous. And then a physicist, Carl Michal analyzed orientation of different amino acids in the silk of Dragline by using NMR technique.[14] Spider's silk contains complex microstructures and outstanding mechanical strength.[15] The recombinant silk of spider gives a relationship between their mechanical properties with their proteins. [16] They contain biopolymers of protein. For e.g. Spider Aranae's silk is constructed with the help of many types of protein designs and their microstructures.[17]

The most unexceptional property of spider silk is that it is as strong as steel as and finer than human hair. [18]And the Dragline silk is meant to be very tough biopolymer on the Earth because of outstanding combination of its elasticity and strength. [19] It has great uses in biomedicine, biotechnology and transplant operation.[20]Their strength originates from their proteins nature,[21] but the spider silk consist of very low density. [22] Unfortunately, large amount of silk cannot be obtained from spiders easily, so other than animals, the recombinant silk is

produced by taking the help of transgenic potato and tobacco plants. [23] Dragline silk and egg case silk have composition of amino acid similar to silk which is produced by orb weaving spiders.[24]

Different categories of silks can also be produced by the spiders. They all are of different types according to their composition of amino acids. [25] And that is why it is a challenge to put efforts on producing recombinant silk by biotechnological techniques. [26] Genetically modified spider goats produce milk having spider silk in it. [27] The dragline silk have high elasticity and tensile strength. This combination makes dragline silk mechanically very powerful as it is 5 times stronger than any other silk of spider. It consists of matrix which is filled with nano-crystal like particles, but till yet their exact structure is not determined. By the help of X-ray diffraction, it has been seen that nano-crystals are oriented with polypeptide chains paralleled whereas alanine residues perpendicularly. This organization gives silk unique mechanical property.

Dragline silk have less advantage because its proteins are quite large and hard to solubilize after formation of silk fibers whereas recombinant silk have regular sequence and can be easily purified and solubilize. [28]

APPLICATIONS

They have range from adhesives, cocoons to orb web construction. [29] Spider silk supports the adhesion and also the growth of bladder cells. Bladder reconstruction by the help of synthetic and natural material is a challenge because of mechanical failure type of adverse effect. Spider silk have slow degradation rate so it helps in growth of multipotent bladder cells. [30] Films, hydro gels, microspheres, nanospheres, microcapsules, fibers and foams can also be made and produced by recombinant spider silk.

At low pH, the spider silk are polymerized and they form hydro gels, whereas at two immiscible phases the silk film forms microcapsules. Spheres are formed by addition of the phosphate ions, then the salting out of silk protein takes place by forming micro and nano spheres.[31] Spider silk is very biocompatible and also biodegradable, therefore it is very helpful in pharmacy such as it is helpful in tissue engineering as well as in drug delivery

systems. [32] Various biomaterials can also be produced and made by the help of spider silk. [19] Biotechnological techniques are there which provides industrial application of silk proteins. [16] It can also be converted to make solid fiber from spider silk protein which results in forming protein based material in medical sciences. [33] Recombinant spider silk AvMaSp-R contains 240 amino acids and it was injected in insect cell infected by baculo virus.

The recombinant spider silk do not activate the macrophages and therefore it provides a non-inflammatory response which shows that it can also be applied for biomedical applications. [34] Hydro gels can be made up of poly anions proteins made up of recombinant spider silk. [35] It is used in implant coatings, wound dressing devices and also in making surgical threads. [36] Silk proteins of 7 different types are known of orb weaver species of spiders. [36] Each of them differs in their physical properties, the protein sequence and their functions. [19]

HOW IT IS DONE

Animals are more appreciated bioreactors than the recombinant cell cultures because they consist of great metabolic pathways, they are reproducible, and they can be easily maintained. The recombinant proteins are produced mammalians milk as it offers flexible production.[33] The European Union state transgenesis process of organisms as they are the genetically modified organism, for e.g. animals, microorganisms and plants. Their genetic properties are modified artificially.

In practice this means to insert or delete the information present in nucleus's DNA of single cell. And then they are then getting multiplied for forming a proper organism which will be named as genetically modified organisms. To develop a transgene, there are various techniques such as DNA microinjection, injection of sperm intra cytoplasmic, retroviral vectors and nuclear transfer of somatic cell. When the goal is to produce proteins, then the DNA coding is encoded into the target/host genome.

To accomplish this expression, the transgene should consist of a promoter, insulator introns enhancers, and terminator. The important use of protein expression in the animals is getting the yield of pharmaceutically important proteins. By using, the promoters from genes, in the milk protein the biopharmaceuticals can be easily

obtained.[30] Proteins derived from spider silk proteins i.e., silk fibroin showed outstanding biocompatibility, customized properties and very easy processing.[39]the protein sequence of spider silk protein which are engineered was obtained from naturally occurring spider silk protein ADF4, which was produced by Araneusdiadematus'

European garden's spider, to use it as dragline silk fiber.[32] In addition of new character, the introduction of the genetic material can be used as to silence the genes. It is done by the help of expressing synthetic oligonucleotides with the sequence of mRNA of targeted gene. Due to this the translation is prevented. It can also be done by expressing intracellular antibodies against the target protein.[33] There are 3 methods which are based on DNA and thus they are applicable for identification of genetically modified organisms.

1) Screening methods- it targets on the DNA sequences which are inserted in genetically modified organisms for e.g. promoters, terminators. It does not allow to detect with accurate certainty that which genetically modified events are present in sample.
2) Construct Specific methods- It targets on three or many other genetic elements in a transgene. These methods can detect absence or presence of events but it does not distinguish between different events.
3) Event Specific methods- It targets the host transgene DNA junction. These methods are accurately and highly specific and also they help in are confirmation genetically modified positive samples.[33]

DETAIL OVERVIEW

The recombinant silk protein' molecular weight ranges from 71 to 700.01 kDa which depends on source. cDNA clones encodes the two different dragline silks which are then isolated and analysis is done by taking the help of any two species of N.clavipesorb web weaving spiders, and Araneusdiadematus.[34]

The extraordinary strength of the spider silk is because of hydrophobic and hydrophilic regions, also in combination with the chain orientation achieved at the time of spinning [34]

To provide control self-alignment, the author suggested that beta-sheet structures of silk, sequence of spider silk was changed

with the poly alanine sequence [34] The R5 peptide is derived from silaffin protein of the diatom Cylindrothecafusiformis, which forms nanostructures that can be reproduced and precipitation of silica by taking the help of silicic acid obtained from specie silaffins, was added into the silk to generate nano composites for bone regeneration . For a new genetic delivery systems, ampiphilic block type of copolymers based on silk are developed with poly(l-lysine) to give a good transfection efficiency through endocytosis which is integrin-mediated[35].

These types of designs are used for the control of size for gene delivery, stability and required in the needs for gene delivery. The recombinant based silk-like polymers demonstrate it as very advanced highly modified biomaterials with many types of features from the composite material to the gene delivery.

Silk Proteins' advantages As Biomaterials in Drug Delivery Systems

The delivery of certain molecules which are biocompatible and also bioactive at the site of action. Other than this such type of delivery system can also be more helpful if the product would be biodegradable, having good biocompatibility, also in nature it is mechanically durable and they are prepared or processed under very aqueous conditions to retain bioactivity of the medicament which has to be delivered. Silks which can be useful in addressing these requirements, because of the properties and characters like self-assembly, bio-degradability, bio-compatibility processing flexibility, and mechanical toughness. Various studies shows that the silk of spider is a lot biocompatible and also less inflammatory if compared with the common biodegradable polymers for e.g. poly (lactide) [35]

The other important feature of silk is their processing ability into different material format, for e.g. as films, hydrogels, nanoparticles and nanofibers[36].

Uses of Recombinant Spider Silks in Drug Delivery Systems

Recombinant proteins of spider silk-

The proteins of recombinant dragline silk which are obtained from the spider named Araneusdiadematusare also useful to make microcapsules for the drug delivery by the help of using its one of the property i.e. self-assembly for all the proteins at the time of interface of emulsion. These capsules are also estimated to be useful for encapsulate the small active ingredients. Microspheres of

bioengineered types of spider silks, which are derived from ADF4 from the A. diadematus, can also be produce by other methods for e.g., dialysis and micro mixing. [36]

CONCLUSION

The gene pharming technique will help the world in better way of treating the wounds and as it supports weak blood vessels so it will be very useful in making artificial tendons or ligaments. So far in this article we tried to focus on the spider goat, how the spider's silk can be produced in goat's milk by the use of transgenesis, which is named as recombinant silk protein.

Their applications in pharmaceuticals will provide a vast development in treating different types of ailments and also preparing films, foams, hydrogels etc. Recombinant silk protein can be made up of three methods i.e. screening method, Contrast Specific method, and Event Specific method. Its protein is biodegradable and biocompatible therefore it can also be used in areas like tissue engineering and also drug delivery systems. According to a survey, the recombinant spider silk protein will be of great value until the end of this century.

ACKNOLEDGEMENT

Authors acknowledge the support and motivation from Management of SVKM's NMIMS, our Director Pharma Institutions Dr. R. S. Gaud sir and Associate Dean Dr. A. S. Deshpande madam.

CONFLICT OF INTEREST STATEMENT

Author declares no conflict of interest

REFERENCES

1. Humphreys JM, Chapple C, 2000. Molecular 'pharming' with plant P450s. Trends Plant Sci; 5:271–2. Doi: 10.1016/ S1360-1385(00)01680-0.
2. Lillico SG, McGrew MJ, Sherman A, Sang HM, 2005. Transgenic chickens as bioreactors for protein-based drugs. Drug Discov. Today 10:191–6. Doi:10.1016/ S1359- 6446 (04) 03317
3. Houdebine LM, 1994. Production of pharmaceutical proteins from transgenic animals. J Biotechnology; 34:269–87.
4. Levy MF, 2000. Animal organs for human transplantation Proc (Bayl Univ Med Cent); 13:3–6.
5. Spider Silk Service RF, 2002. Materials science. Mammalian cells spin a spidery new yarn. Science; 295:419–21. doi:10.1126/science.295.5554.419b.

6. Lazaris A, Arcidiacono S, Huang Y, Zhou JF, Duguay F, Chretien N, 2002. Spider silk fibers spun from soluble recombinant silk produced in mammalian cells. Science; 295:472 doi: 10.1126/ science.1065780.
7. Agnarsson I, Kuntner M, Blackledge TA, 2010. Bio-prospecting finds the Toughest Biological Material: Extraordinary Silk from a Giant Riverine Orb Spider. PLoS One; 5:e11234. Doi: 10.1371/ journal.pone.0011234.
8. Houdebine LM, 2009. Production of pharmaceutical proteins by transgenic animals. Comp Immunol Microbial Infect Dis; 32:107–21. Doi: 10.1016 /j.cimid.2007.11.005.
9. Gould P, 2002. Exploiting spiders' silk. Mater Today; 5:42–7. Doi: 10.1016/ S1369- 7021(02)01238-5.
10. Lewis† R V. Spider Silk: Ancient Ideas for New Biomaterials 2006. Doi: 10.1021/ CR010194G.
11. Vendrely C, Scheibel T, 2007. Biotechnological Production of Spider-Silk Proteins Enables New Applications. Macromol Biosci; 7:401–9. Doi: 10.1002/ mabi.200600255.
12. Hu X, Vasanthavada K, Kohler K, McNary S, Moore AMF, Vierra CA, 2006. Molecular mechanisms of spider silk. Cell Mol Life Sci 63:1986–99. Doi: 10.1007/s00018-006- 6090-y.
13. Winkler S, Kaplan DL, 2000. Molecular biology of spider silk. J Bio technol; 74:85–93.
14. Tokareva O, Michalczechen Lacerda VA, Rech EL, Kaplan DL, 2013. Recombinant DNA production of spider silk proteins. Microb Bio technol; 6:651–63. Doi: 10.1111/ 1751-7915.12081.
15. Chung H, Kim TY, Lee SY. 2012. Recent advances in production of recombinant spider silk proteins. Curr Opin Bio technol 23:957–64. Doi: 10.1016/ j. cop bio .03.013.
16. Teulé F, Cooper AR, Furin WA, Bitten court D, Rech EL, Brooks A, 2009. A protocol for the production of recombinant spider silk-like proteins for artificial fiber spinning. Nat Protoc; 4:341–55. Doi: 10.1038/ nprot.2008.250.
17. Widhe M, Johansson J, Hedhammar M, Rising a, 2012. Current progress and limitations of spider silk for biomedical applications. Bio polymers; 97:468–78. doi:10.1002/bip.21715.
18. Scheller J, Gührs KH, Grosse F, Conrad U, 2001. Production of spider silk

proteins in tobacco and potato. Nat Biotechnol; 19:573–7. Doi: 10.1038/ 89335.

19. Casem ML, Turner D, Houchin K 2005. Protein and amino acid composition of silks from the cob weaver, Latrodectus hesperus (black widow). Int J Biol Macromol 24:103–8.
20. Brooks AE, Lewis R V, 2004. Probing the elastic nature of spider silk in pursuit of the next designer fiber. Biomed Sci Instrum; 40:232–7.
21. Guhrs KH, Weisshart K, Grosse F, 2000. Lessons from nature--protein fibers. J Biotechnol; 74:121–34.
22. Oroudjev E, Soares J, Arcidiacono S, Thompson JB, Fossey SA, Hansma HG, 2010. Segmented nanofibers of spider dragline silk Atomic force microscopy and single-molecule force spectroscopy. 27:245–52
23. Kluge JA, Rabotyagova O, Leisk GG, Kaplan DL, 2008. Spider silks and their applications. Trends Biotechnol; 26:244–51. Doi: 10. 1016/ j.tibtech. 02.006.
24. Steins A, Dik P, Müller WH, Vervoort SJ, Reimers K, Kuhbier JW, 2015. In Vitro Evaluation of Spider Silk Meshes as a Potential Biomaterial for Bladder Reconstruction. PLoS One; 10:e0145240. Doi: 10.1371/ journal. pone.0145240.
25. Kaźmierska K, Florczak A, Piekoś K, Mackiewicz A, Dams Kozłowska H, 2011. Engineered spider silk: the intelligent biomaterial of the future. Part II]. Postepy Hig Med Dosw 65:389–96.
26. Spiess K, Lammel A, Scheibel T, 2010. Recombinant Spider Silk Proteins for Applications in Biomaterials. Macromol Biosci 10:998–1007. Doi: 10.1002/ mabi.201000071.
27. Heim M, Keerl D, Scheibel T, 2009. Spider Silk: From Soluble Protein Extraordinary Fiber. Angew Chemie Int Ed; 48:3584–96. Doi: 10.1002 /anie.200803341.
28. Lee KS, Kim BY, Kim DH, Jin BR, 2016. Recombinant spider silk fibroin protein produces a non-cytotoxic and non-inflammatory response. J Asia Pac Entomol 19:1015–8. Doi: 10.1016 /j.aspen.2016.09.004.
29. DeSimone E, Schacht K, Scheibel T, 2016. Cations influence the cross-linking of hydrogels made of recombinant, polyanionic spider silk proteins. Mater Lett; 183:101–4. Doi: 10.1016/ J.MATLET.2016.07.044.

30. Schacht K, Scheibel T. Processing of recombinant spider silk proteins into tailor made materials for biomaterials applications. Curr Opin Bio technol 2014; 29:62–9. Doi: 10.1016/ J.COPBIO. 2014.02.015.
31. Verma PK, Kundu A, Puretz MS, Dhoonmoon C, Chegwidden OS, Londergan CH, 2018. The Bend Libration Combination Band Is an Intrinsic, Collective, and Strongly Solute- Dependent Reporter on the Hydrogen Bonding Network of Liquid Water. J Phys Chem B;122:2587–99. doi:10.1021/acs.jpcb.7b09641.
32. Livens A, Petrillo M, Querci M, Patak A, 2015. Genetically modified animals: Options and issues for traceability and enforcement. Trends Food Sci Technol; 44:159–76. Doi: 10.1016/ J.TIFS.05.001.
33. Hofer MM, Hofer M, 2013. Development of spider silk protein particles for pharmaceutical applications, 1–196
34. Slotta UK, Rammensee S, Gorb S, Scheibel T, 2008. An Engineered Spider Silk Protein Forms Microspheres. Angew Chemie Int Ed; 47: 4592–4. Doi: 10.1002/ anie.200800683.
35. Scheibel T. Spider silks, 2004. recombinant synthesis, assembly, spinning, and engineering of synthetic proteins. Microb Cell Fact; 3:14. Doi: 10.1186/1475-2859-3-14.
36. Nagal A, Singla RK, 2013. Applications of Silk in Drug Delivery: Advancement in Pharmaceutical Dosage Forms. Indo Glob J Pharm Sci; 3:204–11.