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An Introduction to Recombinant Proteins-A Review

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Abstract

Native and recombinant proteins benefit major sectors of the biopharmaceutical industry, the enzyme industry, and the agricultural industry and its development is visibly increase in recent years. Vaccines, insulin, human growth factor, digestive enzymes and monoclonal antibody are examples of proteins produced with recombinant technology. As we see, the importance of recombinant protein is clear from its products. In this review, we tried to simply introduce recombinant protein technology. First of all recombinant DNA technology introduced which lead to sudden development of recombinant protein technology. In the next part Monoclonal Antibodies, Enzyme production, Elastin, collagen, and fibrous described in details.
Key world: Recombinant protein, DNA, Antibodies,agricultural industry,Pharmaceutical

Introduction

These days many proteins for pharmaceutical or other specialized purposes are produced through biotechnological methods. Many different human proteins like insulinT growth hormone, vaccines and also some proteins used in diagnostic tests could produce in abundance for therapeutic uses. To illustrate, by using a harmless bacteria like *E.coli* which contain a human insulin gene, insulin could produced easily in the bacteria culture. This insulin can be purified and used for treating diabetic people. Digestive enzymes are another interesting proteins which could produce in microorganisms. These engineered cells could be colonized in the intestinal

tract of peoples who suffer from digestive enzymes insufficiencies (1). Recombinant DNA technology caused to a great development in understanding the molecular basis of diseases. By this tool, we are able to diagnose the existing diseases and also predict the risk of developing a given disease in feature. Gene therapy for sickle cell disease, adenosine deaminase deficiency and some other disease may be helpful (2). Artificial blood vessels produced from collagen tubes coated with layers of the anticoagulant heparin is the product of modern biotechnology. Proteins are the most part of biotechnological products and nearly all of them must isolate from proteinceous substances. So proteinceous is the most common cause of recombinant drugs impurities. These impurities sometimes caused to allergic reactions or may change the therapeutic effects of drug. Only a slight difference between recombinant protein and its

Tab. 1 Short time line in pharmaceutical biotechnology(4)

No	Historic event	Year
1	Jenner inoculates child with viral vaccine to protect him from smallpox	1797
2	Pasteur proposes that microbes cause fermentation	1857
3	Penicillin is discovered by Fleming	1928
4	Avery proves DNA as carrier of genetic information	1944
5	Waksman isolates streptomycin as antibiotic for tuberculosis	1944
6	Structure elucidation of double helix of DNA	1953
7	First protein sequencer is perfected	1967
8	Discovery of restriction enzymes	1970
9	Cohen and Boyer produce first recombinant DNA in bacteria with restriction enzymes and ligases	1973
10	First expression of human protein in bacteria	1977
11	US Patent for gene cloning to Cohen and Boyer	1980
12	First transgenic animal	1981
13	Humulin ® as first recombinant biotech drug approved by FDA	1982
14	Invention of Polymerase Chain Reaction (PCR)	1983
15	First recombinant vaccine for Hepatitis B (Recombivax HB ®)	1986
16	First US Patent for genetically modified mouse (Onkomouse)	1988
17	Launching of the Human Genome Project	1990
18	First somatic gene therapy to cure ADA-SCID	1990
19	First transgenic cow produces human proteins in milk	1990
20	Approval of DNAse for cystic fibrosis	1994
21	First animal cloned from adult cell (Dolly)	1997
22	Rough draft of the human genome is announced	2000
23	Draft version of the complete map of the human genome is published	2002
24	First oligonucleotide drug is approved by FDA	2002

endogenous counterpart can elicit immune response. In the case of bacteria cultures, contaminants of nitrogen-containing bacteria can elicit an adverse immune responses. The occurrence of contamination in the manufacture products of traditional cell cultures is as much as the recombinant cultures. In any of them, low level of microbial contamination in cultures is easily detectable (3). Biotechnology is the art of using scientific and engineering principles together for processing and changing materials with biological agents to new good ones.By the expanding of genetic engineering, biotechnology brings to the forefront of science in society. By the early 1978s, with production of the first synthetic human insulin, biotechnology rapidly grew. By the 1988, only five proteins from engineered cells had been approved by the Unites States Food and Drug Administration (FDA), but by the end of 1990s, this number reached to 125 proteins (table 1).

Recombinant DNA

Peter Lobban was the first one who proposed the idea of recombinant DNA (5). The first articles describing successfully production and intracellular replication of recombinant DNA were published in 1972 and 1973 (6,7). Recombinant DNA (rDNA) molecules are some kind of DNA formed by genetic engineering methods in laboratory by which a sequences is created that would not be found in biological organisms. The key point which make recombinant DNA possible is that DNA molecules from different organisms share the same chemical structure (figure 1).

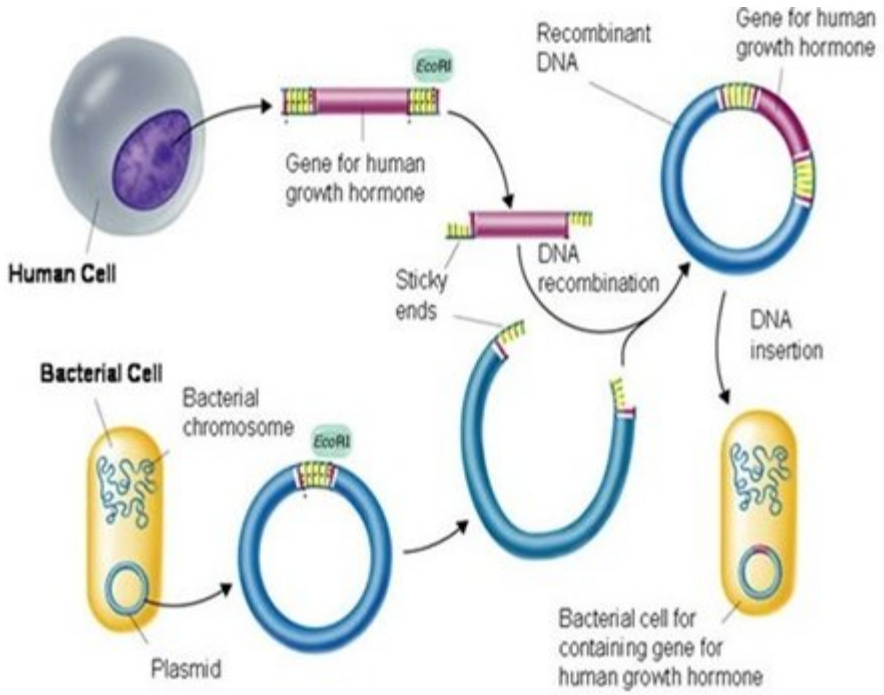


Figure 1: Construction of recombinant DNA, in which a foreign DNA fragment is inserted into a plasmid vector

Expression of recombinant DNA

After transferring foreign DNA into the cell, this gene may or may not be expressed. This means thet, the DNA may replicated without any expression or may also translated after transcription and so produced a recombinant protein. In order to express a gene in a foreign cell, it need to restructure the gene for induction of sequences which are require for mRNA production by host's translational apparatus like promoter, signal of translation initiation (8). Also in some cases, specific changes in host organism may be need to improve its gene expression or changes may be need at coding sequences for translation optimization, making soluble protein, and stabilization of protein from degrading enzymes (9).

Applications of recombinant protein

Pharmaceutical products

Most of traditional drugs are small molecules that activate or deactivate a biological process through binding to their targets. These molecules mainly produced through traditional organic synthesis. But in contrast, other larger molecules like proteins which developed for addressing targets that small molecules cannot, are biopharmaceutical drugs. Here we mention some of these drugs: Infliximab ,a monoclonal antibody used in autoimmune disease treatment, Etanercept, also used in autoimmune treatment and is a fusion protein and Rituximab which is another antibody for anti cancer properties. Biopharmaceutical are big molecules and for this and also because of their difficulty surviving in stomach, colon, and liver, they typically injected. Modern biotechnology and genetic engineering are linked together for the production of like synthetic proteins. *E.coli* and sa*ccharomyces cerevisiae* are two cells which most of genetic manipulations performed on them. It can also do on animals and plants and produced transgenic animals or plants. For example Chinese Hamster Ovary cells (CHO), are used for manufacturing certain pharmaceuticals(Table 2).

Recombinant protein-based polymers for advanced Drug delivery:

With regard to advances in recombinant technology, genetically engineered polymers with exquisite control over monomer sequence and polymer length has been developed.By studying precise structures correlate with function this is possible to use these polymers for drug delivery. By using chemical methods, many useful polymers for drug delivery manufactured. But there is some limitations include statistical characterization of conventional polymer synthetic techniques. It's possible to produce such polymers with specific function and physicochemical properties in organisms. For example according to elastin-like, silk like and silk-elastin like protein polymers , delivery systems developed with developed physicochemical properties. Also production of protein based polymers is growing up and it will produce promising constructs with the ability to transfer gene or drug. There is no report of using recombinant polymers for bioactive agents delivery, but the information obtained from previews researches with these polymers can be used for designing more safe and effective polymers for clinical uses (10,11)(figure 2).

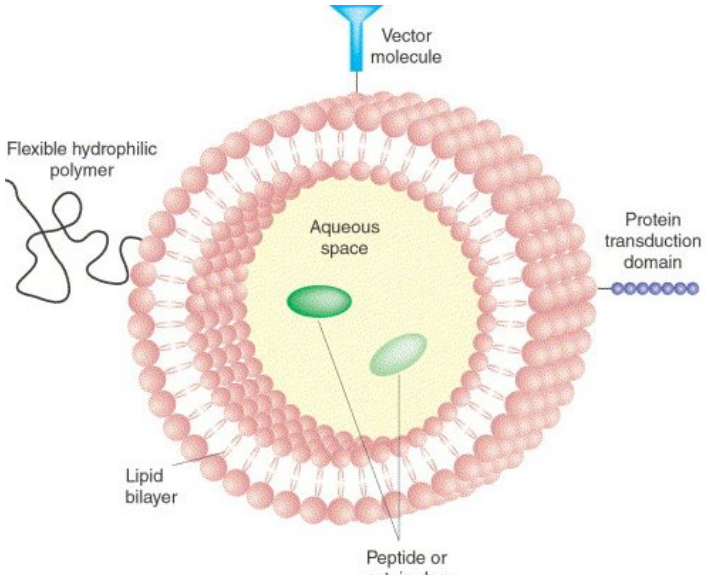


Figure 2: The liposome as a vehicle for protein and peptide drug delivery**.** Flexible hydrophilic polymers [for example, poly(ethylene glycol), PEG] increase circulation half-life by modifying the liposome surface. Tumor targeting of the carrier can be enhanced by attaching vector molecules (e.g. antibodies) to the surface and intracellular delivery can be facilitated by the attachment of protein transduction domains. These peptides target the plasma membrane, allowing the delivery of peptides and proteins in a receptor-independent manner (12).

Production of Recombinant Antibodies

Monoclonal Antibodies

Paul in beginning of 20th century proposed the idea of '' magic bullet'' for the first time. This idea said that if a compound be able to selectively target against disease causing organism, so this agent of selectivity could be used for delivery of a toxin for that organism. He received the 1908 Nobel prize for his medical works which led to an effective syphilis treatment by 1910. The first searches for studying the structure of antibody began in 1970s, after this finding that in B-cell cancer multiple myeloma, all cancerous B-ells produce a single type of antibody, but production of identical antibodies specific to an antigen was not yet possible. Georges Köhler, César Milstein, and Niels Kaj Jerne shared the Nobel Prize of 1984 in physiology or medicine for their practice and discovery of monoclonal antibody production method. long life of myeloma cells, specific antibody producing B-cells and successfully selection of healthy producing B-cells are three key points of this invention. Monoclonal antibodies are the same specific antibodies made by identical immune cells of one parental clone, and polyclonal antibodies are different specific antibodies to an antigen produced by different immune cells.

Enzyme production

By introduction of microbial enzymes in 1980s and 1990s, the enzyme industry growth up suddenly. By the 1970s, most enzyme production relayed on traditional extraction from plant or animal sources, which had some drawbacks like: high prices, low availability, and impurity. Microbial enzymes were a good alternative for previous ones. They were economically favorable since microbial cultivation was cheaper, simpler and faster than plants or animals and their manipulation was so easy. Mutagenesis and random screening of microorganisms helped scientists for production of higher enzymes titers. The advantages of using recombinant enzyme technology are:1) microbial fermentations can easily produce plant and animal enzymes, 2) now days production of enzymes from species with difficulty in growth or genetically handling is possible in microorganisms , 3) using multiple gene copies, strong promoters and efficient signal sequences caused to increase in enzyme production 4)by using protein engineering stability, activity and/or specificity of an enzyme improve. In 1993, recombinant processes produced more than 50% of industrial enzymes (13,14). *Aspergillus niger* engineered for plant phytase production, prepared food for more than 50% of pigs in Holland. By using this recombinant cells, 1000 fold increase in phytase production was achieved (14, 15). Lipase was industrially produced by A.Oryzae which is used for laundry cleaning, glucosides esterification and glycolipid production for surfactants, skin care products and many other uses .mammalian chymosin was another enzyme cloned in A.niger and also E.coli and the interesting thing about recombinant chymosin was its price which was half of its natural type.

Recombinant protein scaffolds for tissue engineering:

By the appearance of strategy to restore damaged tissues, tissue engineering faced with new area of treatment. This technology includes culturing autologous or allogeneic cells like stem cells for forming functional tissues for implantation. The scaffold is an important part for this type of culture because, it provide a support for cell adhesion and proliferation, and also used for some materials needed for mimicking natural extracellular matrix of human body. The final aim of tissue engineering technology is to restore or induce tissue regeneration. (16).

Elastin and elastic proteins

The mechanical integrity and elasticity of mammalian tissues is mainly because of some proteins like Elastin. Elastin is an important component of several tissues. For example, more than 50% of aorta, 30% of other vessels and many other tissues are Elastin. There are many different proteins which may have elastic properties. Elasticity referred in some cases to the ability of reversibly deformation while this term is also used for the ability of extending to large strains with lower energy consumption. In the case of Elastin, a combination of these two properties seen (16).

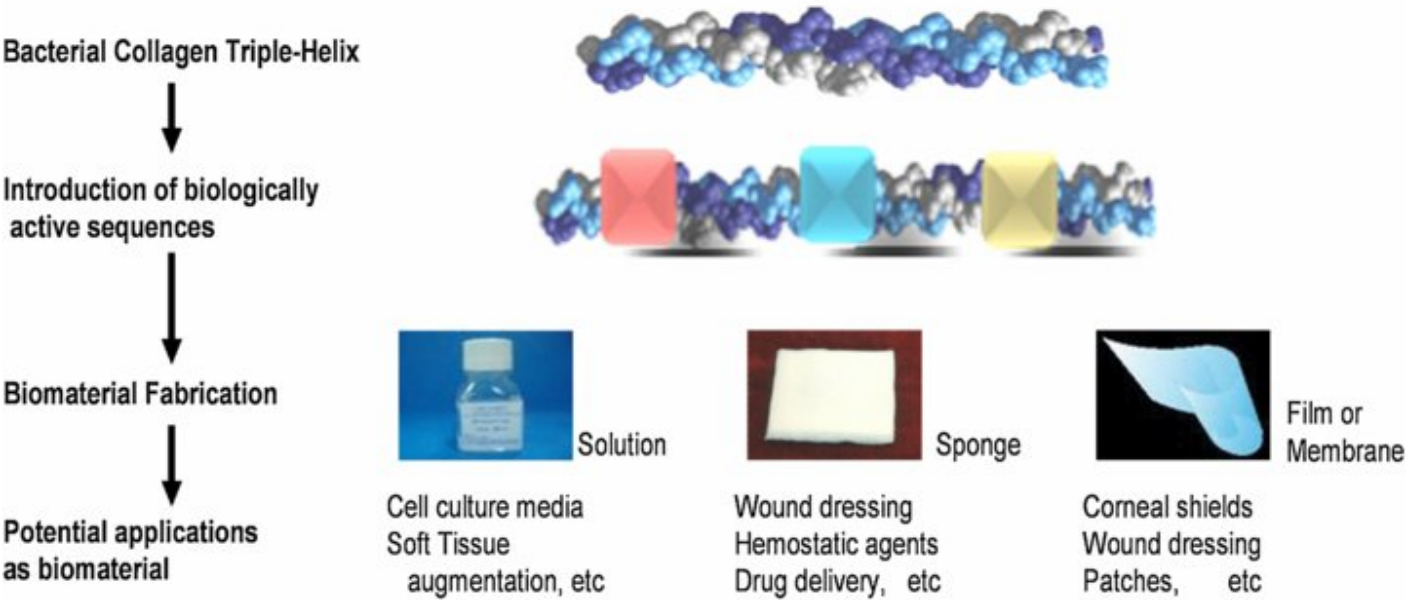
Collagens

Collagen is another useful example of recombinant technology which is used in biomedical industry (Figure 3). This protein mainly presented in connective tissues include skin, bon, cartilage and tendon (16).

Fibrous

Fibrous proteins, with their importance in tissue engineering, are gaining increased attention. They are one of the oldest medical materials. Keratin, was used as an material used for surgicalsuturing in earliest medical text (16).

Figure 3. Design and fabrication of multi-functional recombinant collagen biomaterials (Werkmeister and Ramshaw, 2012).



Conclusion

Different hosts can be used for recombinant protein production in variety culture conditions. Depending on type of host, method of culturing, extraction and purification method the costs of protein manufacturing differ. By develops in protein engineering, purification methods and cloning tools, recombinant proteins are being closer to shelves. But lots of researches are still needed for more qualified products and producing proteins more easily.

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Table. 2 Therapeutic Biologic Products Approvals (without Diagnostics) (17,18).

No	Trade Name	Proper Name	Approval Date
1	Santyl	Collagenase	1965
2	Elspar	Asparaginase	1978
3	Intron A	Interferon alfa-2b	1986
4	Intron A	Interferon alfa-2b	1986
5	Roferon A	Interferon alfa-2a	1986
6	Activase, Cathflo Activase	Alteplase	1987
7	Epogen	Epoetin alfa	1989
8	Neupogen	Filgrastim	1991
9	Leukine	Sargramostim	1991
10	Proleukin	Aldesleukin	1992
11	Betaseron	Interferon beta-1b	1993
12	Pulmozyme	Dornase alfa	1993
13	ReoPro	Abciximab	1994
14	Oncaspar	Pegaspargase	1994
15	Avonex	Interferon beta-1 a	1996
16	ProstaScint	Capromab Pendetide	1996
17	Rituxan	Rituximab	1997
18	Infergen	Interferon alfacon-1	1997
19	Neumega	Oprelvekin	1997
20	Zenapax	Daclizumab	1997
21	Regranex	Becaplermin	1997
22	Retavase	Reteplase	1998
23	Simulect	Basiliximab	1998
24	Synagis	Palivizumab	1998
25	Synagis (Liquid)	Palivizumab	1998
26	Verluma	Nofetumomab	1998
27	Remicade	Infliximab	1998
28	Enbrel	Etanercept	1998
29	Herceptin	Trastuzumab	1998
30	Ontak	Denileukin diftitox	1999
31	Actimmune	Interferon gamma-1b	1999
32	TNKase	Tenecteplase	2000
33	MYOBLOC	RimabotulinumToxinB	2000
34	Aranesp	Darbepoetin alfa (polysorbate solution)	2001
35	PegIntron	Peginterferon alfa-2b	2001
36	Aranesp	Darbepoetin alfa (albumin solution)	2001

37	Campath	Alemtuzumab	2001
38	Kineret	Anakinra	2001
39	Humira	Adalimumab	2002
40	Pegasys	Peginterferon alfa-2a	2002
41	Elitek	Rasburicase	2002
42	Neulasta	Pegfilgrastim	2002
43	Zevalin	Ibritumomab tiuxetan	2002
44	Rebif	Interferon beta-1a	2002
45	Fabrazyme	Agalsidase beta	2003
46	Aldurazyme	Laronidase	2003
47	Xolair	Omalizumab	2003
48	Bexxar Therapeutic Regime	Tositumomab and Iodine I-131	2003
49	Avastin	Bevacizumab	2004
50	Copegus Combination Pack	Peginterferon alfa-2a	2004
51	NeutroSpec Technetium	Fanolesomab	2004
52	Kepivance	Palifermin	2004
53	Erbitux	Cetuximab	2004
54	Botox	Onabotulinum Toxin Type A	2005
55	Naglazyme	Galsulfase	2005
56	Botox Cosmetic	Onabotulinum Toxin Type A	2005
57	Vectibix	Panitumumab	2006
58	Elaprase	Idursulfase	2006
59	Myozyme	Alglucosidase alfa	2006
60	Soliris	Eculizumab	2007
61	Mircera	Methoxypolyethylene glycol epoetin beta	2007
62	Nplate	Romiplostim	2008
63	Cimzia	Certolizumab pegol	2008
64	PegIntron	Peginterferon alfa-2b and Ribavirin	2008
65	PegIntron	Peginterferon alfa-2b and Ribavirin	2008
66	Amevive	Alefacept	2008
67	Arcalyst	Rilonacept	2008
68	Kalbitor	Ecallantide	2009
69	Arzerra	Ofatumumab	2009
70	Dysport	Abobotulinum Toxin Type A	2009
71	Extavia	Interferon Beta-1b	2009
72	Simponi	Golimumab	2009
73	Raptiva	Efalizumab	2009
74	Stelara	Ustekinumab	2009
75	Ilaris	Canakinumab	2009
76	Prolia	Denosumab	2010
77	Xgeva	Denosumab	2010
78	Xeomin	IncobotulinumtoxinA	2010
79	Xiaflex	Collagenase Clostridium Histolyticum	2010
80	Krystexxa	Pegloticase	2010

81	Actemra	Tocilizumab	2010
82	Lumizyme	Alglucosidase alfa	2010
83	Nulojix	Belatacept	2011
84	Benlysta	Belimumab	2011
85	Orencia	Abatacept	2011
86	Yervoy	Ipilimumab	2011
87	Campath	Alemtuzumab	2011
88	Sylatron	Peginterferon alfa-2b	2011
89	Adcetris	Brentuximab vedotin	2011
90	Xigris	Drotrecogin alfa (Activated)	2011
91	Eylea	Aflibercept	2011
92	Xigris	Drotrecogin alfa (Activated)	2011
93	Erwinaze	asparaginase Erwinia chrysanthemi	2011
94	Voraxaze	Glucarpidase	2012
95	Raxibacumab	Raxibacumab	2012
96	Jetrea	ocriplasmin	2012
97	Granix	tbo-filgrastim	2012
98	Orthoclone OKT3	Muromonab-CD3	2012
99	Zaltrap	ziv-aflibercept	2012
100	Lucentis	Ranibizumab	2012
101	Perjeta	Pertuzumab	2012
102	Kadcyla	ado-trastuzumab emtansine	2013
102	Alferon N Injection	Interferon alfa-n3 (Human Leukocyte Derived)	2013
104	Simponi Aria	Golimumab	2013

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