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Biopharmaceuticals: New yet Natural

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Authors' contributions

This work was carried out in collaboration between both the authors. Author NS designed the outline of the manuscript. Both the authors contributed equally to the literature search, draft preparation and self evaluation of the manuscript. Both the authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Biopharmaceuticals are large complex therapeutic drug molecules composed of amino acids, nucleotides and proteins obtained by extraction from non-conventional sources. The biopharmaceutical products include cytokines, interleukins, enzymes, hormones, monoclonal antibodies and clotting factors. Biopharmaceuticals due to their complex heterogeneity are highly sensitive and unstable in nature and may undergo degradation if temperature or pH is altered. The term "biopharmaceutical" is still debatable and the definition of biopharmaceutical is still unclear. The current review intends to throw a light on the general information regarding biopharmaceuticals such as their regulatory definition(s), types, production systems (microbial, plants and mammalian), characterization and delivery techniques of biopharmaceuticals. The global scenario of the sales of biopharmaceuticals is represented along with a case study of biopharmaceuticals in cancer treatment and the sales of oncological products world over. The Indian biotech industries have grown immensely in the last few years such that various pharmaceutical industries are diversifying into biopharmaceutical production. In contrast to so many therapeutic applications, the biopharmaceuticals possess few limitations such as bioaccumulation in the body, toxicity, immunogenicity, contamination, high manufacturing cost etc. The biopharmaceuticals hold a promising future for the healthcare industry. Many innovations in biopharmaceuticals are underway including biosimilars, biobetters, biodrugs, diagnostic biomarkers such as lab on a chip technology and drug therapy based on genetic buildup of an individual (Pharmacogenomics).

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Keywords: Biopharmaceuticals; biosimilars; biologics; cytokines; recombinant DNA technology; monoclonal antibodies; oncological products; Lab on a chip.

1. INTRODUCTION

Over the past four and a half decades, Biotechnology has contributed immensely to the healthcare system. Since the discovery of recombinant DNA technology in the 1970s to the launch of the Ist recombinant human insulin, the healthcare system progressed by leaps and bounds towards the better treatment and management of a wide range of previously incurable diseases. Several biopharmaceutical drugs such as proteins, peptides, monoclonal antibodies have been introduced into the market since then. The biopharmaceuticals have grown from 13 in 1989 to 210 in 2012 [1]. The biopharmaceuticals are obtained from the non conventional sources (different from pharmaceuticals which are obtained from conventional sources, either naturally or synthetically) by carrying out modifications in the biological source. The flow chart in Fig. 1 depicts the sources of both pharmaceuticals and biopharmaceuticals.

1.1 Defining Biopharmaceuticals

The definition of biopharmaceuticals is still unclear and no clear cut classification criteria exist till date to put them in a defined category. In the view of Ronald A Rader, a biopharmaceutical is "a pharmaceutical inherently biological in nature and manufactured using biotechnology" [2]. There are various definitions of biopharmaceuticals depending upon the biological source (microbial, mammalian or plant) and nature of the product to those based on business models. The biopharmaceuticals are not directly extracted from biological sources but obtained by making slight modifications in the source.

1.2 Regulatory Definitions of Biopharmaceuticals

The regulatory agencies such as the United States Food and Drug Administration (USFDA) and European Union (EU) have defined biopharmaceuticals based on their paradigm of broad biotechnology view and new biotechnology review [2].

USFDA defines biopharmaceuticals under the term 'biologics' or 'biological products'. The FDA definition of biologics may be recapitulated as "derived from living material- human, plant, animal, or microorganism – and used for the treatment, prevention or cure of diseases in humans" [3]. The biological products include "a wide range of products such as vaccines, blood and blood components, allergenics, somatic cells, gene therapy, tissues, and recombinant therapeutic proteins" [4].

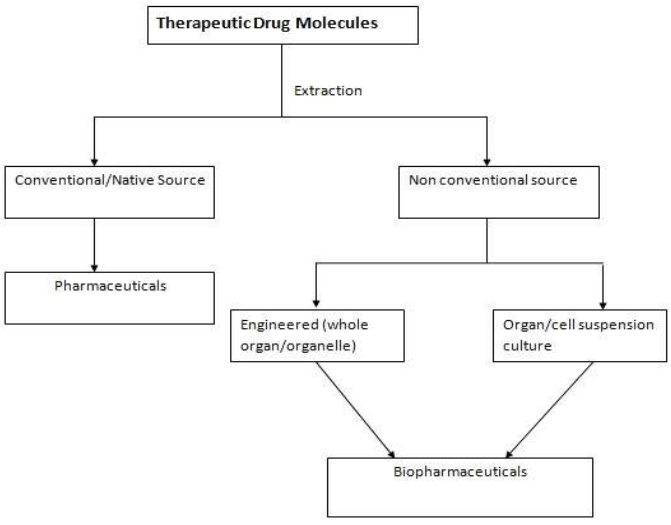

Fig. 1. Sources of pharmaceuticals and biopharmaceuticals

European Union defines biopharmaceutical as "a protein or nucleic acid based pharmaceutical substance used for therapeutic or *in vivo* diagnostic purposes, which is produced by means other than direct extraction from a native (non-engineered) biological source"[5].

1.3 Proposed Definition of Biopharmaceuticals

We as authors propose a source and technology based definition for biopharmaceuticals as "the therapeutic drug molecules extracted via non-conventional methodologies involving engineered life forms or cell/tissue/organ culture mediated techniques."

1.4 Comparison of Conventional Drugs and Biopharmaceuticals

Biopharmaceuticals are different from pharmaceuticals or chemical-based drugs. Drugs are small synthetic chemical substances which can be developed consistently using standardized processes. Biopharmaceuticals are large complex molecules with greater molecular mass (two to three times as compared to conventional pharmaceuticals) composed of amino acids, nucleotides or chemical subunits. Biopharmaceuticals possess considerable heterogeneity depending upon the source and manufacturing process. These include post translational modifications and differences in the 3D-conformation, infolding of peptide chains, aggregation of molecules, disulphide linkage, oxidation and amidation. All these in turn affect the identity, stability, safety and efficacy of the final product. The products of one manufacturer are unique from the comparable product of another and sometimes variation maybe found even in the different batches by a single manufacturer [2].

Chemical compounds/Pharmaceuticals are defined as small homogeneous drug molecules obtained from synthetic sources. A detailed comparison of small vs. large drug molecules based on various parameters such as their nature, size, molecular weight etc. is given in Table 1.

Table 1. Comparison of small drug molecules (pharmaceuticals) and large drug molecules (biopharmaceuticals)

Parameters	Small drug molecules/ pharmaceuticals	Large drug molecules/ biopharmaceuticals
Physicochemical parameters		
Nature/source	Synthetic	Biological
Size	Single to few molecules	Large biomolecules
Homogeneity	Homogeneous	Heterogeneous
Transport across membrane	Easy and consistent	Inhibited due to their large size
Molecular weight	0.5-1 kDa	>1 kDa
Formulation stability	Mostly stable	Highly sensitive and unstable
Mode of administration	Mostly oral in the form of solids (tablets, capsules) and liquid (syrup, suspension, emulsion)	Given directly into the systemic circulation mostly through parenteral route
Post administration parameters		
Immune response	Mostly Non-Immunogenic	Immunogenic
Synthesis/production	Chemical	Living cell culture
Stability	Stable in living system	Unstable and highly susceptible to degradation
Distribution	Throughout the body	Plasma and extracellular space
Characterization	UV/Vis, IR, HPLC, LC/MS, NMR, DSC	Bioassay, Circular dichroism, capillary electrophoresis, NMR, DSC, Analytical Ultracentrifugation
Half life	Short(<24 hours)	Long(from hours to weeks)

Abbreviations- kDa-kilo Dalton, UV/Vis-Ultraviolet visible spectroscopy, IR-Infrared spectroscopy, HPLC-High performance liquid chromatography, LC/MS-Liquid chromatography mass spectrometry, NMR- Nuclear Magnetic Resonance, DSC- Differential Scanning Calorimetry; Source- References [6,7]

2. TYPES OF BIOPHARMACEUTICALS

Most of the biopharmaceuticals are proteinaceous in nature and are classified into following groups -

2.1 Cytokines

Cytokines are soluble proteins/glycoproteins molecules which carry out the process of cellular communication through signal transduction and play a major role in the development of cellular and humoral immune responses by activating immune cells (lymphocytes and macrophages). These are highly potent substances, produce effects at very less concentrations (nanomolar to picomolar). The administration of cytokines is useful in the treatment of infections by enhancing body's immune response and the manipulation of cytokine activity can help in the treatment of a wide range of diseases [8].

Cytokines possess the following properties –

- These are pleiotropic, i.e. the effect of same cytokine will be different on different cells. e.g. Interferon-γ stimulates the proliferation of T cells, B cells, NK (Natural Killer) cells and macrophages but shows weak anti-proliferative activities with some other cells.
- They exhibit redundancy, i.e. different cytokines may produce a similar effect. e.g. Interleukin-2, Interleukin-4 and Interleukin-5 all stimulate proliferation of B cells as part of their function.
- They perform synergistic functions along with one another. e.g. Interleukin-4 along with Interleukin-3 promotes the proliferation of mast cells [8].
- Some cytokines promote antagonism of effects produced by others. e.g. Interleukin-4 antagonizes many effects of interferon-γ such as inhibition of the cytolytic potential in human Th1 cells (a subtype of helper T cells) clones while Interferon-γ promotes it [9].

Cytokines are grouped into the following categories:

2.1.1 Interferons(IFN)

These are the cytokines which induce anti-viral and anti-proliferative activity in the cells. All interferons produce their effect by binding to specific receptors and initiating signal transduction events which result in the altered expression of IFN-responsive genes thereby producing the physiological effect [8]. They are used in the treatment of several diseases such as leukemia, hepatitis B and genital warts [10]. Some FDA approved interferons are Intron A (interferon alpha-2b) and Pegasys (Pegylated interferon) for the treatment of hepatitis B and Infergen (interferon alphacon-1) for hepatitis C.

2.1.2 Interleukins

Interleukins are a group of cytokines which act as messengers between leukocytes. They promote the development and proliferation of B-, T- and hematopoietic cells [10].

2.1.3 Tumor Necrosis Factor (TNF) family

The TNF family activates signaling pathways for regulation of inflammation, cell survival, death and differentiation. They also show cytotoxic activity against tumor cells [10].

2.1.4 Granulocyte- colony stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF)

These cytokines stimulate the bone marrow to produce neutrophils and macrophages which enhance the immune response against infections [10].

2.1.5 Hematopoietins

These are the hematopoietic growth factors responsible for stimulation of erythropoiesis (production of red blood cells).

2.1.6 Hormones

Hormones are the group of regulatory molecules performing vital physiological functions in the human body. The first recombinant hormone synthesized was human insulin in 1982 (Humulin) for the treatment of diabetes mellitus. Before this, insulin was extracted from the animal sources (pigs and domestic animals). But this insulin was slightly different from the human insulin and possessed potential side effects such as induction of immune responses. After this several hormones such as recombinant growth hormone (rGH) and recombinant erythropoietin α have been synthesized using rDNA technology [10]. Other recombinant approved enzymes are Glucagen (recombinant glucagon) for use in hypoglycemia, Thyrogen (thyrotrophin-α) in the

detection or treatment of thyroid cancer and Gonal F (recombinant follicle stimulating hormone) for anovulation and superovulation [11].

2.2 Enzymes and Therapeutic Proteins

Enzymes are proteinaceous compounds, specific in nature which carry out various biochemical reactions in the body. Deficiency of particular enzyme leads to the development of diseases. Enzymes are produced by using recombinant DNA technology by the insertion of gene of interest in a microbial host such as *E. coli* and allowing the expression of enzymes in host cells followed by purification to obtain the desired product [12]. Several enzymes are being widely used for therapeutic purposes in the treatment of diseases like cancer, cystic fibrosis, digestive disorders etc. Some of the FDA approved enzymes are Fabrazyme (Agalsidase beta) in the treatment of Fabry's disease, Adagen (Pegademase) in enzyme replacement therapy in SCID (severe combined immunodeficiency), Oncaspar (Pegaspargase) for the treatment of acute lymphocytic leukemia, Cerezyme (Imiglucerase) for replacement therapy in type I, II and III Gaucher's disease [13]

2.3 Vaccines

The conventional vaccines used consist of killed or attenuated microorganisms which when administered into the body stimulate the immune system to generate antibodies. These antibodies destroy the foreign antigens and protect against infections. These vaccines are generally harmless, however, they possess a risk of the attenuated microorganisms returning to its virulent/pathogenic states and may cause more harm than good [10].

To overcome this risk, recombinant or subunit vaccines were developed by incorporating the genes encoding for the protein portion of the microorganism into a suitable host and expression of the encoded protein under controlled conditions. e.g. HBsAg (hepatitis B surface antigen) protein is the antigenic marker for hepatitis B virus. The HBsAg is expressed in yeast *Saccharomyces cerevisiae* and the protein produced is then extracted from the yeast. The HBsAg polypeptide obtained is thus used for immunization against hepatitis B [14]. Other recombinant vaccines which are in the developing phase are against HIV and Ebola.

Plants have also emerged as a source of antibody production. The plantibodies or recombinant antibodies produced in transgenic plants are undergoing extensive research for the reason that plants are the most inexpensive source and less prone to contamination [15]. Plant cells assemble and fold antibodies similar to mammalian cells [16]. CaroRx is a chimeric IgA/G produced in transgenic tobacco plant and is undergoing clinical trials [15].

2.4 Monoclonal Antibodies

The antibodies produced by the immune system in response to a particular antigen are varied and are termed polyclonal whereas monoclonal antibodies (mAb) are derived from single clones of hybridoma cells (the cells formed by the fusion of antibody-producing B cells and myeloma cells), which target for a single specific epitope. The monoclonal antibodies produce both properties of continuous growth of the myeloma cell and antigen specific properties of the B cell [17].

The monoclonal antibodies due to their ability to bind to specific receptors on tumor cells with high specificity mediate cytotoxic action on the tumor cells. The various monoclonal antibodies presently in the market are Trastuzumab (Anti-Her 2, humanized mab) for treatment of metastatic breast cancer, Rituxan (Anti CD20 IgG1, chimeric mab) for leukemia, Avastin for treating colorectal cancer, breast cancer, kidney cancer etc [18].

2.5 Clotting Factors

The clotting factors are essential for the efficient completion of the clotting process. Lack of clotting factors may result in hemophilia [10]. Examples of recombinant clotting factors approved by USFDA are NovoSeven (Coagulation factor VIIa), Benefix (Coagulation Factor IX) and Tretten (Coagulation Factor XIII A-Subunit) [19].

3. PRODUCTION OF BIOPHARMA-CEUTICALS

The biopharmaceuticals are manufactured using recombinant DNA technology. The product should have appropriate three-dimensional structure and undergo post translational modifications in order to maintain its activity, safety and efficacy. The cell based expression

systems are the most suitable choice but they have their own limitations. These are complex systems and there is no complete control over the protein expression and secretion of the product from the cells. The products may vary from culture to culture depending upon the intrinsic factors e.g. select genotype, inoculum size, culture density and extrinsic factors e.g. temperature, pH, aeration, ionic strength, concentration of micronutrients. These factors together may have marked influence on the protein expression, folding, glycosylation and stability [6]. Thus the cellular expression system is optimized to get a consistent and high yield of protein and further purification of the product is carried out using analytic or specific separation techniques.

The bioreactors are used for the production of biopharmaceuticals on large scale. The main objective of biopharmaceutical production process is to obtain the therapeutic protein in maximum quantity so as to commercialize the process. In the past few years, there has been a tremendous increase in the number and demand of biopharmaceuticals obtained from animal cell culture. But the production process is complex and requires more understanding for scale-up [20].

There are three types of cell production systems for bioreactors as described in Fig 2:

3.1 Microbial Cells

The microbial cells marked the beginning of the production of recombinant drugs. They are easy to cultivate and have short generation time. The first expression platform was produced in *Escherichia Coli* and was widely used host for the production of insulin and human growth hormone. The only problem associated with this expression system was its inability to carry out glycosylation (a form of post translational modification) in the final product [21]. Other microbial systems used are yeast *Saccharomyces cerevisiae* which is used for the production of hepatitis B vaccine by incorporating the viral surface protein HBsAg (Hepatitis B surface antigen) of hepatitis B virus into yeast cells using recombinant DNA technology [14]. The yeasts may express glycosylation of protein product but the pattern may differ as compared to that of humans [21]. The various approved biopharmaceuticals obtained from microbial sources are given in Table 2.

3.2 Mammalian Cells

The mammalian cells such as Chinese hamster ovary (CHO) cells and baby hamster kidney (BHK) cells are the ideal production systems for the manufacture of human therapeutic proteins. These system are able to perform specific post translational modifications of proteins and introduce glycosylation pattern at correct sites [22]. But the glycosylation pattern introduced by the CHO cells are slightly different from that of humans which may lead to generation of immunogenicity and increased clearance of the recombinant product (Ghaderi et al., 2010; Padler-Karavani and Varki, 2011) [23]. The human cell lines are the best choice for the production of these proteins as the folding and post translational modifications would not pose any threat towards developing an immune response. The disadvantage of using mammalian cell lines is their high cost of maintenance and slower growth [22]. Some of the approved marketed products of mammalian origin are mentioned in Table 3.

3.3 Plant Cells

The plants are the most inexpensive source and possess enormous potential for the production of biopharmaceuticals [24]. The plants containing an inserted foreign gene in their DNA are known as transgenic plants. The foreign gene is transferred either by *Agrobacterium* mediated method or by particle gun bombardment. The process of using transgenic plants or animals for the production of biopharmaceuticals is called molecular farming or biopharming [25]. There has been extensive research done in plant biopharming for the development of therapeutic proteins during the last two decades. Although most of the research on plant-made biopharmaceuticals is in the development phase and is undergoing clinical trials, fewer have gained approvals such as first plant-made vaccine against Newcastle Disease Virus (NDV) developed in suspension culture of transgenic tobacco cell lines [26] and the first biological dug taliglucerase α [27]. The major limitations of plant bioreactors are lower yields, downstream processing and non-authentic glycan structures on the recombinant proteins [28]. Plants may be the potential alternative of bacterial, yeast and mammalian cell cultures in the future [16]. Some of the approved products of plant origin are shown in Table 4.

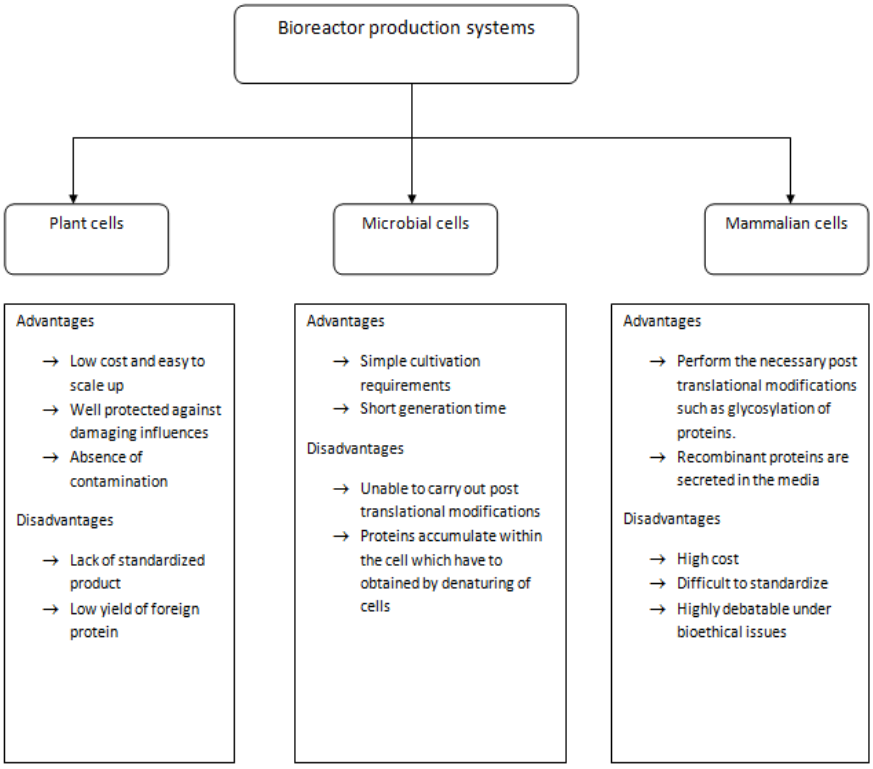

Fig. 2. Bioreactor production systems

Table 2. FDA/ EU approved products of microbial origin

Product /patent number	Patent number	Patent expired Yes(Y) /No(N)	Microbial host	Protein	Company/market authorization holder	Therapeutic applications
Humalog	US 5474978	Y	*E. coli*	Insulin lispro	Eli Lily	Diabetes Mellitus
Nutropin AQ	US 5763394	Y	*E. coli*	rh growth hormone	Schwartz Pharma AZ	Growth failure, turner's syndrome
Miacalcin	US 5733569	Y	*E. coli*	r Salmon calcitonin	Novartis Corporation	Paget's Disease
Tritantrix-HB	US 8431136	N	*S. cerevisiae*	Combinatorial Vaccine containing rHbsAg	Smithkline beecham	Vaccination against Hepatitis B, tetanus, pertussis
Glucagen	US 4826763	N	*S. cerevisiae*	Rh Glucagon	Novo Nordisk	Hypoglycemia
Roferon A	US 5766582	N	*E. coli*	Rh-IFN-α-2a	Schering Corporation	Hairy cell leukemia
Actimmune	EP 069887	Y	*E. coli*	Rh-IFN- α-1b	Genentech	Chronic granulomatous disease

Abbreviations- E.Coli- Escherichia Coli, S.cerevisiae- Saccharomyces cerevisiae, r- recombinant, rh- recombinant humanized, IFN- interferon
Source [Reference Number- 11,29-31]

Table 3. FDA/EU products of mammalian origin

Product	Patent number	Patent expired Yes(Y) / No(N)	Host cells	Protein	Company/market authorization holder	Therapeutic applications
Benefix	US 9062299	N	CHO cells	Rh factor IX	Genetics institute	Haemophilia B
Enbrel	US 7276477 B2	N	CHO cells	rTNFR-IgG fusion protein	Amgen Inc.	Rheumatoid Arthritis
Bioclate	EP 1638491 A1	N	CHO cells	Rh-Factor VIII	Centeon	Haemophilia A
Activase	US 7723573 B2	N	Animal cell lines	Rh-tPA	Genentech	Acute myocardial infarction
Avonex	EP 1082132 A1	N	CHO cells	Rh-IFN-β-1a in CHO cells	Biogen	Relapsing multiple sclerosis
Infuse	US 7435260 B2	N	CHO cells	Rh Bone morphogenic protein-2	Medtronic Sofamor Danek	Promotes fusion of lower spine vertebrae
Xigris	US 20090068721 A1	N	Human cell lines	Drotrecogin-α ; rh activated protein C	Eli Lilly	Severe sepsis

Abbreviations- CHO- chinese hamster ovary, r- recombinant, rh- recombinant humanized, TNFR- tumor necrosis factor receptor, tPA- tissue plasminogen activator, IFN- interferon; Sources- [11,29-31]

Table 4. Biopharmaceuticals of plant origin

Product	Patent number	Patent expired	Host	Company/market authorization holder	Therapeutic applications	Approved (A) / under trials (U)
Locteron (interferon-α)	US 8022270	N	Duckweed	Biolex Therapeutics	Hepatitis C	U
α-1-antitrypsin	-		Rice	Sarcamento, CA	Cystic fibrosis, liver diseases	U
Elelyso	-	N	Carrot	Protalix Biotherapeutics	Gaucher disease	A
VEN100	-		Rice	Ventria Bioscience	Antibiotic-associated diarrhea	U
Herpes Simplex Virus	-	-	Soyabean, Rice	EPIcyte	Therapeutic (potential)	U
Glucocerebrosidase	US 5929304	N	Tobacco	Croptech and Virginia Tech	Gaucher disease	U
CaroRx	TM 75426295		Tobacco	Planet Biotechnology	Dental caries	U

Sources- [27,30,32-34]

4. CHARACTERIZATION OF BIOPHARM-ACEUTICALS

There biopharmaceuticals are characterized using standards mentioned in the Q5C and Q6B guidelines established by the International Council of Harmonization (ICH) and are analyzed for physicochemical, biological and immunological properties [35]. The biopharmaceuticals are also checked for purity, impurity and containments. The various methods used for characterization are-

4.1 Circular Dichroism

Circular dichroism (CD) is an optical spectroscopic technique used in the determination of absolute configurations of optically active molecules by measuring the differential absorption of the left and right handed circularly polarized light (CPL). In case of biopharmaceuticals, CD in the near UV region (300-250 nm) is used to characterize the tertiary structure whereas in far UV region (260-170 nm), it is used to characterize biopharmaceuticals in terms of α-helical, β-strand and undefined secondary structure [36]. When CPL passes through an optically active sample, the differential absorption of the two circularly polarized light components occur and the resulting radiation is elliptically polarized forming an ellipse. The CD spectrum thus obtained is compared with the reference spectrum of the corresponding protein [37].

4.2 Capillary Electrophoresis

Capillary electrophoresis (CE) is a high performance separation technique used in the analysis of biomolecules. In this technique, the samples are analyzed by their ability to travel across the capillary when an electric field is applied. The negatively charged proteins will migrate towards the positive end depending upon their charge and mass. The samples are then analyzed under UV for characterization.. The CE can be employed in combination with mass spectrometry (MS) for even more potent analysis of biopharmaceuticals [38]. CE-MS technique has been used for the analysis of intact protein analysis of recombinant insulin, growth hormone, recombinant interferon-β etc. as reviewed by Pioch et al. [39].

4.3 NMR (Nuclear Magnetic Resonance)

NMR spectroscopy explores the properties of few atomic nuclei (^{1}H, ^{13}C, ^{15}N, ^{31}P) to absorb and re-emit radiation discreetly in the radiofrequency region under the influence of strong magnetic field. NMR provides a wide variety of applications in the discovery, design, characterization, mechanism and toxicity related studies of biopharmaceuticals. It is used in 3D- structure determination of complexes, measuring of rate constants and mapping binding sites of peptides and proteins. The NMR spectra consist of highly resolved peaks or cluster of peaks corresponding to different atoms over a wide range of frequencies. The position or frequency of the peaks describes the chemical shift. Chemical shifts help in defining the atom types, neighboring atoms, bond geometry and types of covalent and non-covalent bonds. The hard to characterize molecules such as **heparin** have been analyzed using NMR. The 3D structure of more than 9500 proteins, peptides, oligonucleotides and polysaccharides have been identified using multi-dimensional NMR spectroscopy and stable isotope labeling (with ^{15}N and ^{13}C) [40].

4.4 Analytical Ultracentrifugation (AUC)

AUC is a biophysical technique used for the characterization of significant biomolecules such as polysaccharides, proteins, nucleic acids, enzymes etc. This method relies on measurement of sedimentation capacity of the biomolecules. The AUC is used to analyse the molecular weight, homogeneity, shape and aggregation capacity of the biopharmaceuticals with themselves or other molecules [41].

4.5 Differential Scanning Calorimetry (DSC)

DSC is used to analyze the folded confirmation of biopharmaceuticals. DSC compares the heat capacity difference (C_p) between the unfolded and folded conformations of proteins which is used for determination of protein stability. Another important application of DSC is the characterization of frozen protein solution and dried protein solids [42].

4.6 Scattering Techniques

When electromagnetic radiation (visible light or X-ray) is incident on the macromolecule, the light is scattered and by measuring the intensity of scattered light the information about the properties such as molecular weight, size, shape, aggregation and higher order structure (HOS) can be obtained [43].

5. DELIVERY OF BIOPHARM-ACEUTICALS

Being proteinaceous in nature, the formulation of biopharmaceutical plays a major role in determining the efficacy of the drug. One of the major challenge lies in the delivery of the biopharmaceutical to the site of action or in systemic circulation. The various approaches

applied for the different routes of administration of biopharmaceuticals have been highlighted by [44]-

5.1 Parenteral

The parenteral route is the most common approach for the administration of biopharmaceuticals because of its ability to redirect the drug directly into the systemic circulation thereby overcoming the first pass metabolism which results in the degradation of the proteins. The administration occurs by subcutaneous, intravenous, intramuscular, intra dermal, intra peritoneal route. The biopharmaceuticals are formulated in the form of microparticles, nanoparticles so as to achieve targeted drug delivery, a longer biologic half life, high cellular transfection efficiency, enhanced bioavailability and improved immunogenicity during vaccination [1,45]. The major limitation of parenteral route is the pain and invasiveness during administration. Examples of FDA approved products given by parenteral route are insulin, heparin etc.

5.2 Pulmonary Delivery

The lungs provide extended large surface area along with bulk blood supply for efficacious drug absorption. The first pass metabolism is avoided and the inhalation is quite less invasive then the parentral route. The proteins are absorbed from the lungs via transcytosis and paracellular transport. The biopharmaceutical formulations used for the pulmonary route of delivery are aerosols or dry powders. Insulin and dornase alfa formulations have been approved for administration via pulmonary.

5.3 Nasal Delivery

Nasal delivery is a self-administered, painless way of delivering the drug directly into the systemic circulation. The nasal mucosa provides high drug absorption per unit surface area. The main limitation of this method is the nasal epithelium which acts as physical barrier for larger molecules particularly peptides and proteins. Chitosan is incorporated along with the biopharmaceuticals to improve its absorption across the epithelial barrier and thereby enhance the bioavailability of the drug. Examples of approved biopharmaceuticals through this route include salmon calcitonin spray.

5.4 Transdermal administration

The delivery of biopharmaceuticals through skin is difficult and considered impracticable owing to the large drug molecules of biopharmaceuticals. Currently investigations are carried out to increase the permeability of the skin to proteins and peptides by the application of ultrasound waves (phonophoresis) or electric field (ionophoresis). Transdermal formulations of insulin and parathyroid hormone are under clinical trials [1].

5.5 Oral Delivery

The orally administered drugs undergo rapid enzymatic degaradtion in the stomach and are directed to the liver where they undergo first pass metabolism. This first pass metabolism leads to the degradation of biopharmaceuticals as they are mainly composed of proteins and peptides. Currently the research is undergoing on the use of absorption enhancers such as fatty acids and bile salts which improve the oral delivery of biopharmaceuticals [1]. Several colloidal carriers such as liposomes, delivery of biopharmaceuticals [46]. These absorption enhancers along with nanoparticles and mucoadhesive devices been successfully used for the oral delivery of insulin in mice and the study is under further investigation.

5.6 Drug Targeting to Brain

The presence of blood-brain barrier (BBB) provides a hindrance towards the delivery of biopharmaceuticals. The peptides and proteins are transported to the brain via blood. Different strategies have been developed to enhance the transport of biopharmaceuticals across the blood brain barrier such as

- By combination of systemic administration with the transient osmotic openings of the blood brain barrier. This technique was initially used for the delivery of chemotherapeutic agents in malignant gliomas
- By receptor mediated transcytosis (e.g. transferrin and insulin) and adsorptive endocytosis (e.g. cationized albumin)
- Using peptide carrier transport systems (such as that of enkephalin, arginine-vasopressin etc.), amino acid carrier systems (glutamate and phenyl alanine etc.), nucleoside carrier systems (cholin

and thiamine), and hexose carrier systems (glucose and mannose)
- Lipidization of the drug by methylation or acylation.
- Use of prodrugs
- By use of colloidal based delivery systems such as liposomes or nanoparticles

6. GLOBAL SCENARIO

Globally, the total sales of biotech products accounted to 117,610 $m in 2012. The biotechnology market of US contributed solely 47% of the total sales with revenue of 65,483 $m. The other major contributors to the sales of biopharmaceuticals are EU with revenue 36,398 $m and Japan with a sales of 8,925 $m. The BRIC nations have also supplemented the biopharmaceuticals market with a sale of 2,857 $m.

6.1 Biopharmaceuticals in Cancer Treatment: A case study

Cancer is a fatal, invasive genetic disease and consists of uncontrolled proliferation, angiogenesis, abnormal growth of cells, decreased apoptosis etc. Cancer is the cause of several thousand deaths each year. According to American Cancer Society, approximately 1,660,290 cancer cases were diagnosed in United States alone in 2013 and about 580,350 mortalities were recorded [18].

To effectively treat cancer, the disease has to be dealt at the genomic level and the mechanism underlying tumor cell growth needs to be explored. The study of cancer at genomic level has been dissipated world over through two important public cancer genomic databases e.g. International Cancer Genome Consortium and The Cancer Genome Atlas. These databases are the outcome of successful completion of human genome project. Several drugs acting against various kinds of tumors have been developed and many are in the development phase. The oncological products of biopharmaceutical origin have reported marked increase in sales in the year 2012 as shown in Table 5. The sales of the oncoproducts and top selling biotech drugs/biopharmaceuticals in 2012 is depicted in Figs. 3-6.

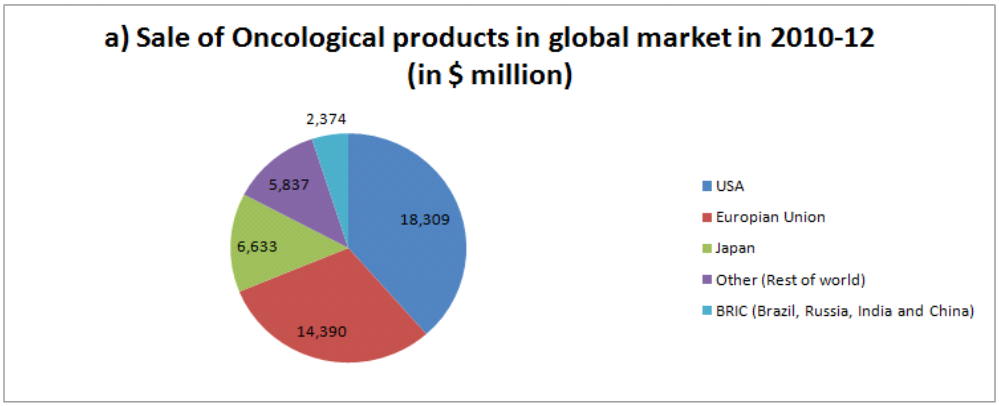

Fig. 3. Sale of Oncological products in global market in 2010-12 (in $ million)

Table 5. Sales of oncological products in the year 2012

S. no	Country	Sales (in $ million)	Compund annual growth rate between 2010-12(%)
1	USA	18,309	+1.2%
2	European Union	14,390	-2.5%
3	Japan	6,633	+5.3%
4	BRIC nations (Brazil, Russia, India and China)	2,374	+1.1%
5	Other (Rest of world)	5,837	+14.3%

Source: Evaluate Pharma [47]

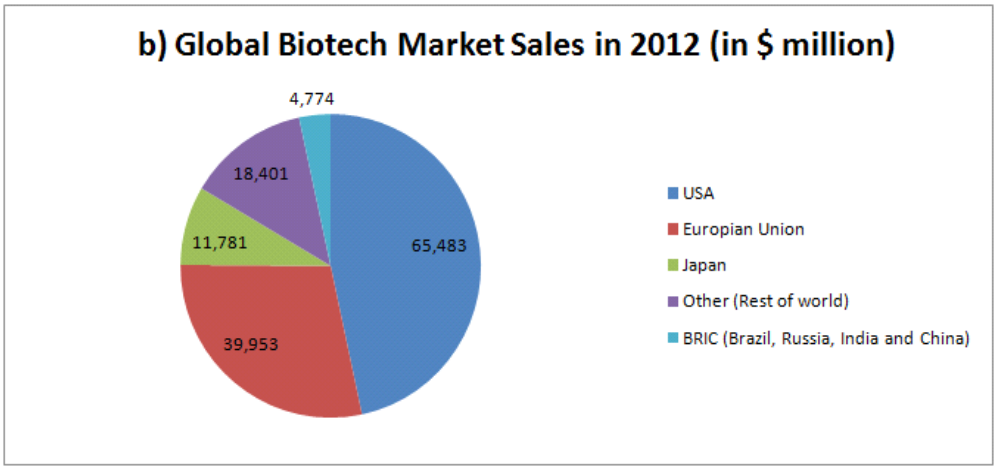

Fig. 4. Global biotech market sales in 2012 (in $ million)

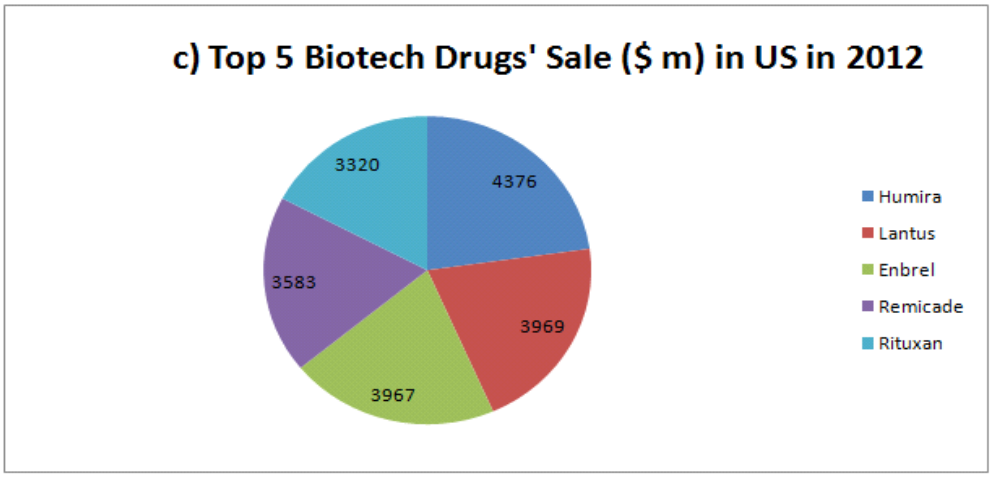

Fig. 5. Top 5 biotech drugs' sale ($ m) in US in 2012

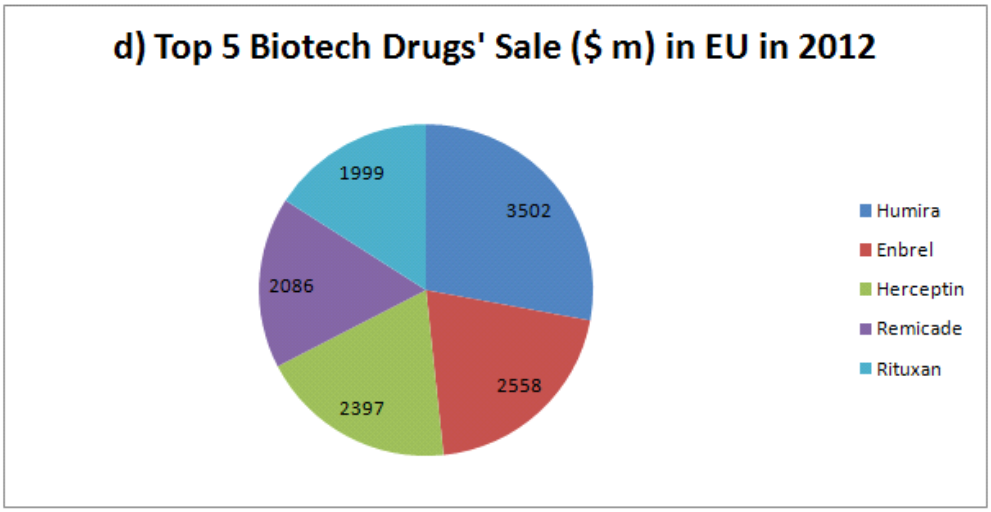

Fig. 6. Top 5 biotech drugs' sale ($ m) in EU in 2012

7. INDIAN SCENARIO

The biopharmaceutical industry in India is one of the fastest developing industries in India with a market size of approx US $2113 million in 2012 to US $2640 million in 2014 [48]. The most of the biotech industries (more than 50%) in India are agriculture based and others are into healthcare and environmental sectors [49]. Many pharmaceutical companies have diversified into biopharmaceutical production. The biopharmaceutical industries mostly belong to the private sector which are being helped by several public research institutions for the standardization of protocols, technology transfer, scale up, testing etc. especially in case of recombinant products. Most of the manufacturers are engaged in the production of biogenerics only. The biogeneric companies are focusing improving the manufacturing process with the motive of fetching patents. For e.g. Bharat Biotech have developed a Caesium chloride free hepatitis B vaccine. Several patents have been filed by the CSIR (Council of Scientific and Industrial Research) institutions [49].

India is also becoming an outsourcing destination for diagnostic testing as the diagnosis is comparatively cheaper as compared to countries such as USA, UK and West Asia. So globally, the countries are outsourcing towards India for diagnostic purposes.

As per the projected data in Fig. 7, Serum Institute of India is top ranking company since 2011-12 amongst the super 10 biotech companies. Prior to it, Biocon was an eternal leader company. Till the year 2010-11, Biocon was ahead of the Serum Institute of India by an annual revenue margin of Rs 442 crores. However in the year 2011-12, Serum Institute of India lead the race by an hopping annual revenue of Rs 1708 crores as compared to Rs 1041 crores. And now since 2011-12, Serum Institute of India is the biotech leader company by a huge annual revenue of Rs 3340 crores as compared to Rs 2218 crores of its nearest competitor Biocon. The reason for sustained leadership of Serum Institute of India is simply based on the production of its H1N1 vaccine (Nasovac) [48].

The potential commercial production of biotech products in India can be well adjudged by comparing the annual revenue of status of top and bottom companies in the list of top 10. For example, in the year 2013-14, Serum Institute of India closed with an annual revenue of 3340 crores while Novozymes South Asia with an annual revenue of 397 crores.

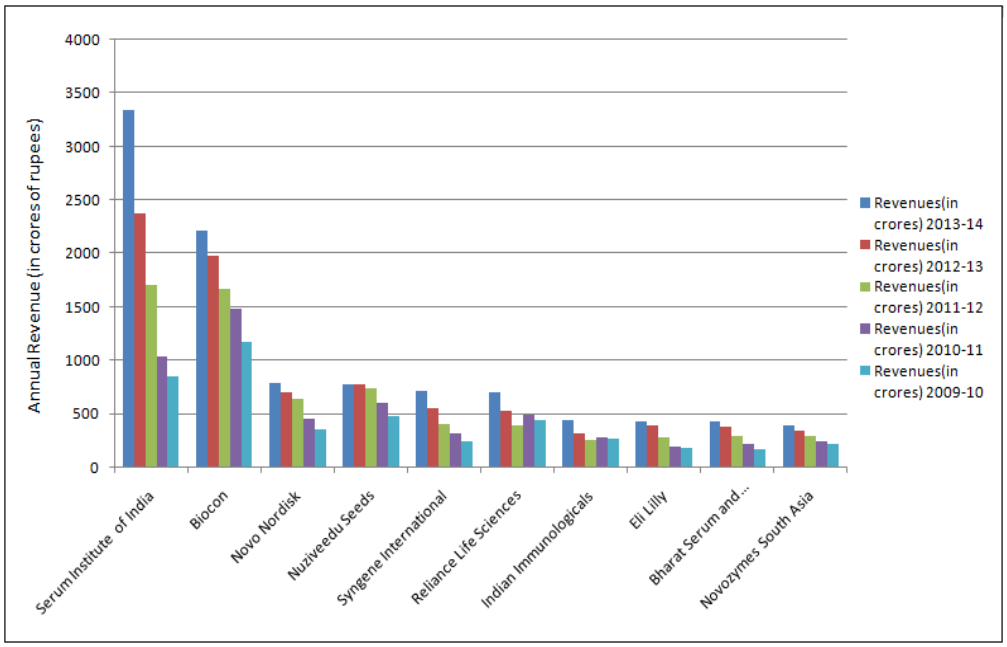

Fig. 7. Annual revenue of Indian biotech companies during 2009-2013

8. LIMITATIONS OF BIOPHARM-ACEUTICALS

- Show therapeutic response at low concentrations, but may cause toxicity at higher concentrations.
- May cause bioaccumulation in the body and may cause damage to the organs.
- More prone to contamination
- Less stability
- High manufacturing costs
- May undergo several modifications in the presence of extreme conditions.
- Requires extensive research and development process and monitoring later on
- Are highly immunogenic
- Sometimes the antibodies produced have different glycosylation pattern from mammalian antibodies.
- Highly debatable and controversial products as they involve transgenic organisms particularly microbes, plants and animals to produce drug molecules [50,51].

9. SAFETY ASSESSMENT /REGULATORY ACTIONS

The safety assessment of biopharmaceuticals involves focusing scientifically and rationally towards the unique characteristics of the product. They are more specific in their action as compared to small drug molecules and have a different safety profile. As the knowledge of the safety profile of biopharmaceuticals at the time of approval is restricted as obtained from preclinical to clinical data, it makes pharmacovigilance studies necessary to be conducted to access any potential side effects related to the use of the biopharmaceutical drug [52].

The International Conference on Harmonization (ICH) has established S6 guideline for the preclinical safety evaluation of Biotechnology-Derived Pharmaceuticals. All three regions i.e. United States, European Union and Japan have followed these guidelines and developed case-by-case scientific approaches for preclinical safety evaluation and market authorization. The primary objective is the identification of a safe dose, target organs for toxicity and the establishment of safety parameters [53].

The safety pharmacological studies are also carried out to investigate any undesirable pharmacological activity of the biopharmaceuticals. Other studies include pharmacokinetics, toxicokinetics, single dose toxicity, repeated dose toxicity and immunotoxicity studies. Further advanced toxicity studies include reproductive performance, developmental toxicity, genotoxicity, carcinogenicity and local tolerance studies [53].

10. FUTURE PROSPECTS

Biopharmaceuticals have contributed immensely in transforming public health issues, increasing longevity and improving quality of life. The interdisciplinary research collaboration between pharmacy and biotechnology has enormously boosted the pharma sector with the more number of pharmaceutical companies now developing keen interest towards development of potential biomolecules.

The whole human genetic code has been sequenced with the completion of Human Genome Project in 2001 consisting of 3.2 billion nucleotide base pairs [1]. The genetic codes and level of gene expression are compared between healthy and diseased individuals for studying the mechanism of the disease progression and developing drug candidates for their treatment. The genomic information obtained from pathogens is being used to identify proteins or genes as targets for drug development.

However there are certain obstacles in the development of biopharmaceuticals owing to their high production cost and greater sensitivity towards the environment. One major challenge is to produce the therapeutic proteins produced in mammalian cell cultures into lower eukaryotic systems. Since the lower eukaryotic cells lack machinery for post translational modifications (such as glycosylation) which determines the pharmacokinetics and immunogenicity produced. So research is going on to modify the eukaryotic genome to produce therapeutic proteins with appropriate post translational modifications [54].

10.1 Biosimilars

Since a lot of top selling biopharmaceuticals are on the verge of patent expiry, many generic drug makers are entering the market with similar products known as biosimilars or follow-on biologics. Some of the patents related to the top selling biologics mentioned in Table 6 are on the verge of expiry which could pave a way for biosimilars.

According to USFDA, " a biosimilar product is a biological product that is approved based on a showing that it is highly similar to an FDA-approved biological product, known as a reference product, and has no clinically meaningful differences in terms of safety and effectiveness from the reference product." [55].

Due to a great conflict amongst biologics, biobetters, biosimilars and me-too products, a sharp decline has been noticed in the newly US and EU approved products i.e. 210 in 2012 and 212 in the year 2014. Out of initially approved 246 products, 34 have already been withdrawn due to various reasons [1,56]. Withdrawl of previously approved products and slower increment in the number is indicative of the fact that presently biopharmaceuticals are getting approval after stringent sieving and screening regulatory norms. Prior to approval from the regulatory agencies, the biosimilars have to come up to the standards of safety and efficacy too. The examples of various biosimilars include Filagrastim, Omnitrope, Silapo etc. [57].

10.2 Biobetters

Generally, all the biopharmaceuticals have short half-lives in the body. Several approaches have been adopted to improve the half-life and thereby improving bioavailability by modifying the biopharmaceuticals resulting in new formulations known as "biobetter drugs". Since the biopharmaceuticals are administered systemically mostly by parenteral route, the biobetters due to their long half-life will have to be administered at longer time intervals than their previous counterparts resulting in improved patient compliance [58].

The biobetters maybe produced by the making the following modifications:

- Glycoengineering i.e. addition of more number of oligosaccharide chains with sialic acid
- Pegylation of the biopharmaceuticals by reaction with monomethoxypolyethylene glycol (mPEG) which is the activated form of polyethylene glycol (PEG). It increases half-life and stability of the drug in body fluid.
- Engineering therapeutic antibodies to form chimeric antibodies to decrease immunogenicity and increase serum half-life
- By fusion of a protein with a biopharmaceutical drug. e.g. fusion of cytokine with a Fc (fragment crystallizable) region of an antibody
- Optimization of the biopharmaceutical using display technology for safety and efficacy. e.g. Adalimumab for treatment of rheumatoid arthritis.

10.3 Biodrugs

The biodrugs are innovative approaches of direct drug production by ingested recombinant microorganisms. The genetically modified microorganisms produce the active drug in the digestive environment. The lactic acid bacteria (Chang & Prakash 1998) and yeasts *Saccharomyces Cerevisiae* and *S.boulardii* which are generally regarded as safe (GRAS) have been studied for this purpose [12].

An innovative biopharmaceutical microbial therapeutics was proposed by Donald et al. (1999). They explained a method of decreasing plasma cholesterol in human body by direct consumption of coprostanol producing microorganisms orally. They were also granted a US Patent vide Patent No. US 5972685 A [patent reference].

The biodrugs hold a massive potential to be explored in the future for the treatment of various diseases such as enzyme deficiencies, infections and control of pro-drug activation etc. [12] But these have to be dealt with biosafety concerns as the genetically modified organisms are ingested into the body, so complete safety profile should be established before qualifying them as drug molecule.

10.4 Diagnostics: Lab-on-a-chip Technology

The biopharmaceutical therapeutics will gain an immense boost if combined with efficacious biomarker based diagnostics. The lab-on-a-chip combines Biotechnology, Nanotechnology, Microfluidics and consists of quick laboratory diagnosis of diseases on a nanoscale in a small amount of time thereby skipping the slow and labor-intensive process. These consist of microchip sized devices made of plastic or glass on which channels are carved. When a drop of blood is placed on one end, it travels down the nano-sized channels where several markers are present which analyze the blood sample and produce diagnostic results [60].

Table 6. Top selling Biologics in 2013 and their patent status [56,59]

Trade name/ description	Company	Treatment	Patent status	
			USA	EU
Humira /TNF Mab	Abott	Rheumatoid Arthritis	Patented till December 2016	Patented till April 2016
Enbrel /TNF Mab	Amgen/Pfizer	Rheumatoid Arthritis, Psoriasis	Patent expired in 2012, second patent granted till 2028	Patent expired in Feb 2015
Remicade /TNF Mab	Johnson & Johnson	Crohn's disease, rheumatoid arthritis, ulcerative colitis	Patented till Sep 2016	Patent Expired in August 2014
Lantus /Insulin Glargine	Sanofi	Diabetes Mellitus	Expired in February 2015	-
Rituxan /CD20 Mab	Roche(Biogen)	Leukemia, autoimmune disorders	Patented till Sep 2016	Patent expired in November 2013
Avastin /VEGF Mab	Genentech(Roche)	Colorectal cancer, breast cancer, kidney cancer	Patented till Jul 2019	Patented till June 2022
Herceptin /HER2 receptor	Roche	Metastatic breast cancer	Patented till June 2019	Patent expired in July 2014
Neulasta /G-CSF	Amgen	Chemotherapy induced neutropenia	Patented till October 2015	Patented till August 2017
Lucentis /VEGF Mab Fab	Roche	Age related macular degeneration	Patented till June 2020	-
Epogen/erythropoietin alpha	Amgen	Erythropoiesis stimulating protein	Patented expired in Aug 2013	Patented expired

The lab-on-a-chip products are already available in the market such as pregnancy test kits and glucose level measuring kits for diabetics. There is a hope for this technology in the future where fatal and difficult to diagnose diseases such as cancer biomarkers will be combined with lab-on-a-chip technology to carry out diagnosis at early stages and thus prompt treatment could be provided to the patient.

10.5 Pharmacogenomics

Pharmacogenomics deals with the study of inherited genetic variations which affect the response of a particular drug in different individuals and to utilize this information to develop drug therapy for individualized population. Single Nucleotide Polymorphisms(SNPs) are used as a diagnostic tool for the prediction of drug response in a person [61].

11. CONCLUSION

Biopharmaceuticals are becoming an important aspect of pharmaceutical industry and possess great futuristic potential to be used as upcoming option for therapeutics. But at the same time, they need to be regulated by various agencies to promote their safer use with least variation and side effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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