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Current Drug Therapy, 2019, *14*, 000-000

REVIEW ARTICLE

Biomimetic Approaches for Targeted Nanomedicine: Current Status and Future Perspectives

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ARTICLE HISTORY

Received: February 20, 2018
Revised: August 18, 2018
Accepted: November 12, 2018

DOI:
10.2174/1574885514666181220092721

Abstract: ***Background*:** Cytotherapy products can be described as "living drugs". Cytotherapy is the swiftest growing fields in the treatment of cancer, heart diseases, aging population and neuromuscular ailments. Biomimetic approaches are processes developed by humans such as devices, substances, or systems that mimic nature or natural processes.

***Objective*:** It aims at developing a base for personalized medicine with allogeneic, autologous and xenogenic therapies where cells are modified for target selection. Such drug delivery methods appear to be complex and challenging. Literature for approximately past two decades was collected and reviewed for the present article.

***Results*:** The opportunities and challenges in cytotherapy have been classified, discussed and demystified. Various process inputs, materials and process conditions required in bioprocessing and preservation have been discussed at length. The review also focuses on the regulatory requirements in India, Europe and U.S.

Keywords: Cytotherapy, bioprocessing, regulatory requirements.

1. INTRODUCTION

Nanotechnology research and initiatives have replaced the concept of conventional drug delivery technology in the past decade, and it is believed that the market of the nano-optimized cellular products would reach around 3 trillion US dollar in 2020 [1]. The prospect of this technology is brilliant as a reasonably young and rapidly developing field. Nanomedicine is achieving realism with nanodiagnostics, drug delivery utilizing nanobiotechnology. Cellular carriers offer advantages over synthetic carriers (polymeric nanoparticles) as these are associated with pulmonary toxicity, systemic effects including blood coagulation, cardiovascular effects [2, 3] and immune adjuvant effects [4].

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Cytotherapy is that subdivision of Regenerative Medicine (RM) industry which is taking rapid strides [5]. It is comprised of immune cell and stem cell therapy; but stem cell therapy makes up a large part of the segment. The global market is motivated by the achievements of stem cell treatments in life-threatening diseases such as heart disease, cancer and neuro-muscular ailments in the aging population. Cytotherapy is expected to lead the market for decades. North America is the leader of the global market since 2015, and is forecasted to remain dominant up to 2022 [6]. Stem cells are there in all multi-cellular organisms; they are primal cells characterized by self-renewal and pluripotency.

Mesenchymal stem cells (MSCs) being multipotent, differentiate into an array of cell types, including adipocytes, chondrocytes, myocytes, osteoblasts, beta-pancreatic islets cells, *etc*. MSCs are of strong therapeutic interest [7, 8] as they represent cells treating a range of acute and degenera-

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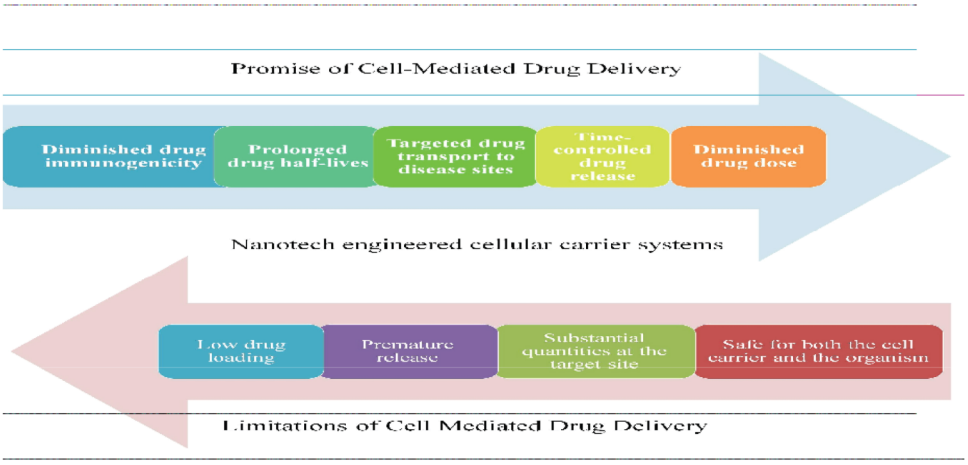

Fig. (1). Potential Opportunity and Challenges to Cell Mediated Drug Delivery.

tive disorders. MSCs are beneficial over other stem cells for many reasons, including their immune-privileged nature making them advantageous for allogeneic transplantation. Their exceptional capacity to convert to structural tissues *via* 3D printing applications is the new field in which MSCs are being explored [9].

Biological cells as drug delivery module enable targeted drug transport, reduction in cell and tissue toxicity and prolonged circulation times (Fig. **1**). Tumor-targeted cell carriers are engineered vectors or special molecules or systems necessarily meant for effective transportation of loaded drug to the desired sites [10].

Cytotherapy technologies and novel methods have already begun to change the tradition of medicine and lead to developing a platform for personalized medicine. Cells are capable of being engineered *via* physical approach, chemical approach, physicochemical and biochemical approach to alter the pharmacokinetic fate and enhance target selectivity. The success of carriers depends on the fitting control of the fate and function of restorative cells. Improved survivability, proliferation, and differentiation of cells are desirable for pro-apoptotic and immune-modulator functioning for cancer treatment [11, 12].

Cellular immunotherapy [13, 14] is a nascent field specifically intended to target brain tumor [15, 16] and particularly beneficial for limiting brain damage and it sets up an enduring antitumor response by stimulating the immune system.

Therapeutic agents for targeting brain tumors need delivery vehicles for the transport of anticancer chemotherapeutics across the blood-brain barrier (BBB) which can be done by different cells, to circumvent toxicity of polymeric carrier systems. They provide, for sustained release, precise drug delivery of enzymatic systems, genetic material to affected organs and tissues. Cell systems hold probable applications in the cure of cancer and other life-threatening diseases.

For adult acute lymphoblastic leukaemia affecting B-cells, the treatment utilizes patients' own T-cells, a type of white blood cells that usually fight viruses and cancer. The patient's blood is rushed through a machine to facilitate extraction of T-cells and restoration of the rest of the blood to the body. Researchers then perform genetic engineering utilizing disabled virus as a "vector" which ferries fresh genetic material to the T-cells, which then reprograms them to identify and kill B-cells that carry CD19 protein on the surface [17, 18].

The goal is to prepare the patients' T-cells to destroy B-cells. Healthy B-cells (which make antibodies to fight infection) are also killed alongside, but the side effect is treatable. More research and clinical trials are nevertheless necessary for the development of cell-based drug platforms for drug delivery [19].

2. CYTOTHERAPY: CLASSIFICATION

1. Autologous or 'one to one' therapy
2. Allogeneic or 'one to many' therapies
3. Xenogeneic cell therapy

2.1. Autologous or 'one to one' Therapy

Defined as those derived from patient's own cells [20]. Autologous cells are readily accepted

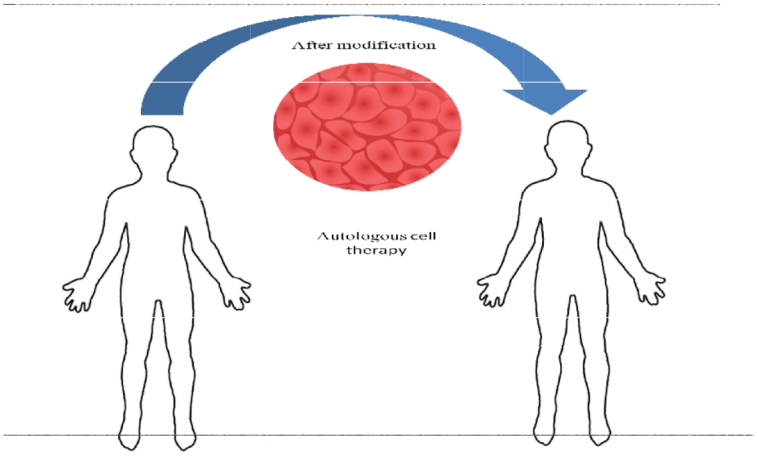

Fig. (2). Treatment with autologous cell after modification.

by a patient, but are unavailable for a lot of treatments. For cell expansion, no large bioreactors or gigantic materials are required and only one small-sized batch is processed in single-use disposables (Fig. **2**). Cytotherapy is used in hospitals, in preclinical settings, and in clinics. Cells obtained from patients are processed locally employing a closed or functionally closed, automated processing system or devices before being reintroduced into the patient, on-site. The finished product is packaged in a sterilized, leak-proof container and shipped from the procurement area to the processing area under guarded conditions maintaining cell viability. The liquid medium in which the specimen is bathed during shipping is optimized to maintain cell and its viability. This kind of therapy does not require scale up [21]. Autologous products present more challenges during processing as lot separation, operational processes and line clearance methods are to be developed to lessen the chances of mix-up of patient-specific lots [22]. The global bioreactor market is growing at a compound annual growth rate (CAGR) of 21.6% during the current period of 2016–2021 and is expected to reach USD 1,085.7 Million in 2021 from the earlier USD 408.4 Million in 2016 [23]. Autologous immune cell-based therapy is now an approved treatment for cancer as per the newly enacted Pharmaceuticals and Medical Devices Agency regulation, Japan [24]. Provenge, the first prostate cancer vaccine was planned to induce immunity against prostatic acid phosphatase (PAP) an antigen mostly expressed in prostate cancers. In provenge, peripheral blood mononuclear cells are cultured with PAP granulocyte-macrophage-colony-stimulating factor (PAP-GM-CSF), a fusion protein consisting of PAP bound to GM-CSF an immune cell activator. During *ex vivo* culturing, PAP-GM-CSF activated antigen presenting cells (APCs) acquire and process recombinant target antigen into peptides that are presented to T-cells. Product categorization shows that PAP and PAP-GM-CSF fusion protein-specific T-cells are generated during healing and are detected in the peripheral blood of patients after treatment with Provenge. It contains a minimum of 50 x 10^6 autologous CD54+ cells which are activated with PAP-GM-CSF, in 250 mL of Ringer's Injection(Lactated). An autologous treatment for certain forms of prostate cancer was found in approximately 25% of cases, that require more than 3 leukapheresis procedures in order to generate the required cell doses, needed for treatment.

It has a shelf life of 18-hour in its insulated container, and when opened, must be thoroughly infused in patients within 3 hours. Provenge is not a preventive vaccine. It is a personalized therapeutic vaccine, administered after prostate cancer has already been diagnosed [25]. A few side effects reported for Provenge include chills, fever, headache, influenza-like illness, myalgia, hypertension, hyperhidrosis, groin pain, rigors, tremor and feeling of coldness [26].

2.2. Allogeneic or 'one to many' Therapies

Allogeneic or 'one to many' therapies utilize cells derived from one donor. The earliest successful allogeneic human hematopoietic stem cell transplant took place in 1968 [27]. The benefit of allogeneic cells is that they do not set off a rejection reaction as harsh as that caused by xenogeneic cells [28]. During production of allogeneic cell-based therapeutics for disorders like heart failure,

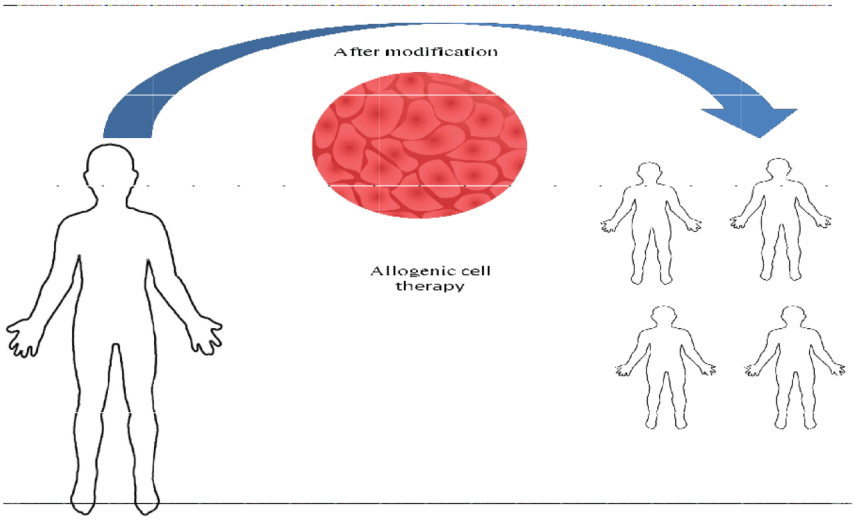

Fig. (3). Treatment with donor cells after modification.

eye diseases, tissue engineering or full organ bio-engineering, there is the need for a huge number (many billions to trillion cells) of functional cells and other requisites *i.e.*, size of the plant, number of workers, number of devices and a number of production rooms. Human allogeneic embryonic stem cell-derived retinal pigment epithelium (RPE) [29], *ex vivo* is capable of being used for cell substitution to restore cell cycle action to support photoreceptors (Fig. **3**). RPE cell substitution is a regenerative therapy which makes up defunct retina cells at the base of the macula and institute a new and healthy layer for the photoreceptors which are losing function because of the old and vanishing RPE layer. It is conceptualized that on replacing the lost RPE layer, the residual photoreceptors start working properly thereby either restricting the decline of the age-related condition or by improving lost visual acuity [30-32]. Prochymal, an allogeneic, bone-marrow derived MSC product which is accepted for management of acute Graft versus host disease (aGvHD) in pediatric patients in Canada, has a shelf life of 2 years at ≤ -135ºC [33].A recent study reports promising trial results of MSC-Frankfurt am Main (MSC-FFM) in therapy for steroid-refractory aGvFD with an overall six-month overall survival probability at an all-time high of 71% as compared to untreated patients [34]. There are no adverse effects or side effects reported for prochymal [34, 35].

2.3. Xenogeneic Cells Therapy

Cells derived from animals such as cows, primates and pigs, are xenogenic cell products. Xenogenic cells are utilized when supplies of human donors are too limited or require characteristics which are not available [36]. It is reported that the use of xenogenic cells has prospective zoonoses effect in humans [37]. Pig islets demonstrate structural and morphological similarities to human islets, and react to sugar levels in the identical physiological range [38]. Human insulin and Porcine insulin varies by only one amino acid, and is used clinically to treat type 1 diabetes patients for many years [39]. Currently, there are no FDA approved xenotransplantation products [40] (Table **1**).

3. CYTOTHERAPY: CHALLENGES AND OPPORTUNITIES

Cellular therapy includes cell and tissue products obtained from biotic material that are employed as 'living drugs.' The 'living drugs' require specialized biological support like bio-preservation, for ensuring viability and structural reliability during handling and *ex vivo* processing. Successful bio-preservation provides optimal recovery, viability, and return to the function of cells, post-preservation to deliver clinical and commercial efficacy. As a new and upcoming research avenue cytotherapy utilizes cells and enables the body to regenerate lost and damaged tissues in many diseases like Parkinson′s, multiple sclerosis, heart disease, liver disease, spinal cord damage, cancer and much more.

Cardiac stem cell (CSC) therapy is clinically used for cardiac repair after myocardial infarction (MI). The manufacturing of cell-based products require some operations and manipulations by in-

Table 1. Summary of cell therapies examined.

Product	Current Owner	Type	Cell Source	Clinical Indication	Nature of FDA Approval(s) and Year(s)
Epicel	Vericel	Autologous	Patient's own skin	Profound dermal burns	Unregulated device (1988)
					Humanitarian use device (2007)
Carticel	Vericel	Autologous	Patient's own cartilage	Cartilage defects	PHS Act, Section 351 (1997)
Provenge	Valeant	Autologous	Patient's own immune cells	Advanced prostate cancer	PHS Act, Section 351 (2010)
Apligraf	Organogenesis	Allogeneic	Skin cells from human foreskin-derived neonatal fibroblasts	Venous leg ulcers (VLU) and diabetic foot ulcers (DFU)	PHS Act, Section 351 (1998 for VLU, 2000 for DFU)
Dermagraft	Organogenesis	Allogeneic	Skin cells from human foreskin-derived neonatal fibroblasts	Diabetic foot ulcers	PHS Act, Section 351 (2001)
Osteocel	NuVasive	Allogeneic	Mesenchymal stem cells & osteoprogenitor cells	Bone regeneration as part of spinal surgery	PHS Act, Section 361
Prochymal	Mesoblast	Allogeneic	Mesenchymal stem cells derived from adult bone marrow	Graft vs host disease	Compassionate Use (2005). Not approved for general use in the US.

dividuals who are well trained in aseptic processing techniques [41].

3.1. Bioprocessing and Preservation of Cytotherapy Product

Formulation for cytotherapy products depends upon various factors like the desired length of storage, transportation factors administration facilities, *etc*. Cell-based biological products are complex and are sensitive to the environment and demonstrate intrinsic variability within a tightly regulated industry. Variation in the product can come from two sources: process input material and process conditions [42]. For allogeneic therapies, most biopharmaceutical companies employ complex collection logistics (Fig. **4**).

Cytotherapy products face several manufacturing challenges [43, 44], for example, they cannot be filtered or sterilized terminally to remove or inactivate microorganism or viruses without affecting the cells. Storage of these products also presents a challenge. To produce a safe and efficient product selection and sourcing of all raw materials is critical as the raw material has the potential of remaining associated with cells used in manufacturing (Fig. **5**).

Preservation of cell lines is desirable from many perspectives [45, 46]. If a graft fails, preservation allows multiple episodes of treatment for the patient with the same batch of cells. Preservation permits autologous cells to be harvested over a period of time, if insufficient cells are collected in a single procedure. Cells are usually cryopreserved in a medium containing DMSO (5%or 10% solution) with or without hydroxyl ethyl starch (6%) and plasma protein such as 4 to 10% human serum albumin in a balanced salt solution or cryoprotective agent (CPAs), which protects the cells from some of the damage caused by freezing [47]. DMSO acts as a stabilizing agent and prevents dehydration of cellular constituents by altering the increased concentration of non-penetrating extracellular solution during ice formation at the time of freezing [48]. The high molecular weight polymeric hydroxyl ethyl solution protects the cells

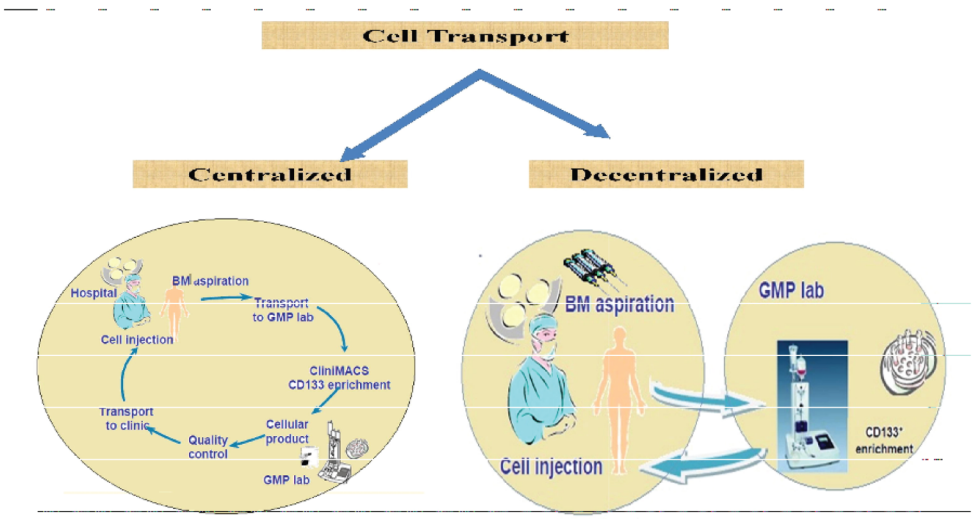

Fig. (4). Cell transfer and Logistics, Perception Centralized *vs*. Decentralized Manufacturing.

from dehydration as water is integrated into the extracellular ice crystals. Maximal recovery and viability of cells can be realized by use of proteins after thawing. Some cryopreserved formulations are entirely free of protein. Freezing is one of the main modes of long term storage, but some cytotherapy products cannot be frozen without change in their characteristics. CPAs are lethal to cells at temperature above 0ºC and a few adverse reactions in patients have been reported when a thawed cell suspension containing DMSO is infused [49, 50] which demonstrates limitations of this approach. An alternative of cryopreservation is hypothermic preservation, maintaining the cells above 0ºC, (short-term protection of cells for some days) [51] significantly slowing down the cell metabolism and they are effectively 'paused'. Hypothermic preservation within the industry (25-33ºC) is used for cell based assays in uncoupling [52] to improve product yield and manufacture of recombinant proteins [53] (Fig. **6**). Chondro Celect (cartilage cells for knee cartilage defects) and Carticel (Genzyme) are formulated in Dulbecco's Modified Eagle's Medium (DMEM) and have a shelf-life of just 48 and 72 hours, respectively when kept under the appropriate storage conditions [54]. These have now been withdrawn from the market after a question from holders of its marketing authorization. Provenge used for prostate cancer is formulated in simple lactated Ringer's Injection, but has a short shelf-life [25]. Post-preservation processing of product (during administration to the patient) depends on the product and method of preservation. Processing steps may include washing and thawing to remove the protective agents used in cryopreservation [55] and dilution to target cell number or simply re-suspension [56] (Fig. **7**).

3.2. Identity

3.2.1. Identity of Cellular Components

Phenotypic and/or genotypic profiling of the cellular components is carried out on the basis of cell population and origin. Identification tests include histocompatibility markers, where applicable, and identification of genetic polymorphisms with specific reference to the intended use.

3.2.2. Identity of Non-Cellular Component

Non-cellular components should be appropriately characterized as such and identity parameters established. Structural components designed to hold the cellular components (scaffolds or membrane) should be identified and characterized with respect to its composition and structural characteristics.

3.2.3. Identity of Combination Products

In a combination product, the active substance may be formed by the integration of cellular and non-cellular components to form a single entity. In this case, the process of combination of the identity of the cellular and non-cellular components may be altered. Consequently, a distinctive way to define identity should be established for the components in the combination, unless justified.

3.3. Cell Purity

The cells of interest for the intended population have certain unrelated characters, often enclosing

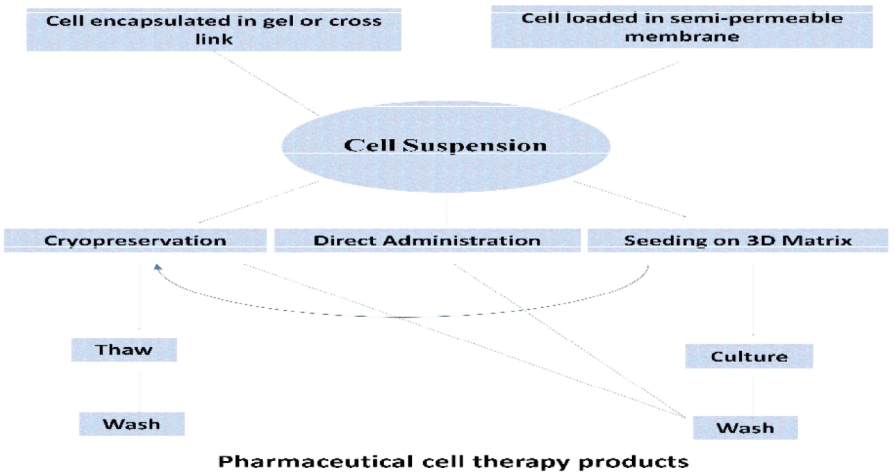

Fig. (5). Processing and Preservation of cell-based Product.

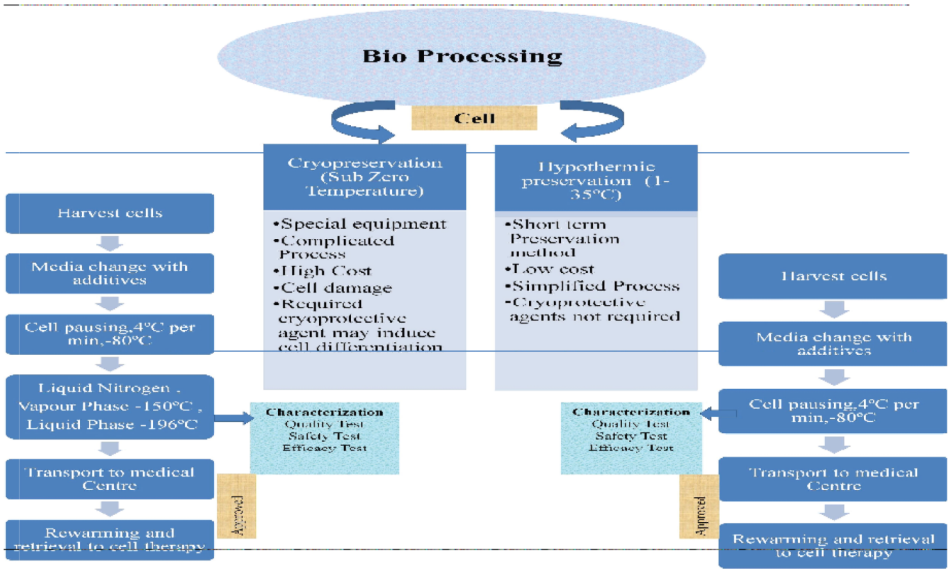

Fig. (6). Flow diagram of cell Bioprocessing.

cells of varying lineages and differentiation stages. Where a particular cell type is required for the indication, the unwanted cells should be defined and their amount in the final product should be controlled by appropriate specifications, *i.e.* acceptance criteria for the amounts of contaminating cells should be set.

3.3.1. Impurities

3.3.1.1. Product or Process-related

During production of cell-based products, many impurities are introduced in the final stages, which could be process and product related. The final product should be analysed for reagents which are identified to be harmful to humans (or in individual components if otherwise not possible) and standard acceptance criterion should be devised. The specification limits should be acceptable by levels detected in batches used for toxicological and clinical studies. Any material which can introduce degradation products into the final product during the production, *e.g.* biodegradable materials, should be thoroughly characterized in this respect and the impact of the degradation products to the cell component(s) should be addressed. If genetically modified cells are used in the product, any additional proteins expressed from the vector, *e.g.* antibiotic resistance factors, selection markers, should be analyzed and there should be proper justification of its presence in the product [57-59].

3.4. Potency

As per the ICH guideline Q6B29 [58], potency is the quantifiable measure of biological activity based on the quality of the product and is linked to the relevant biological properties. The develop-

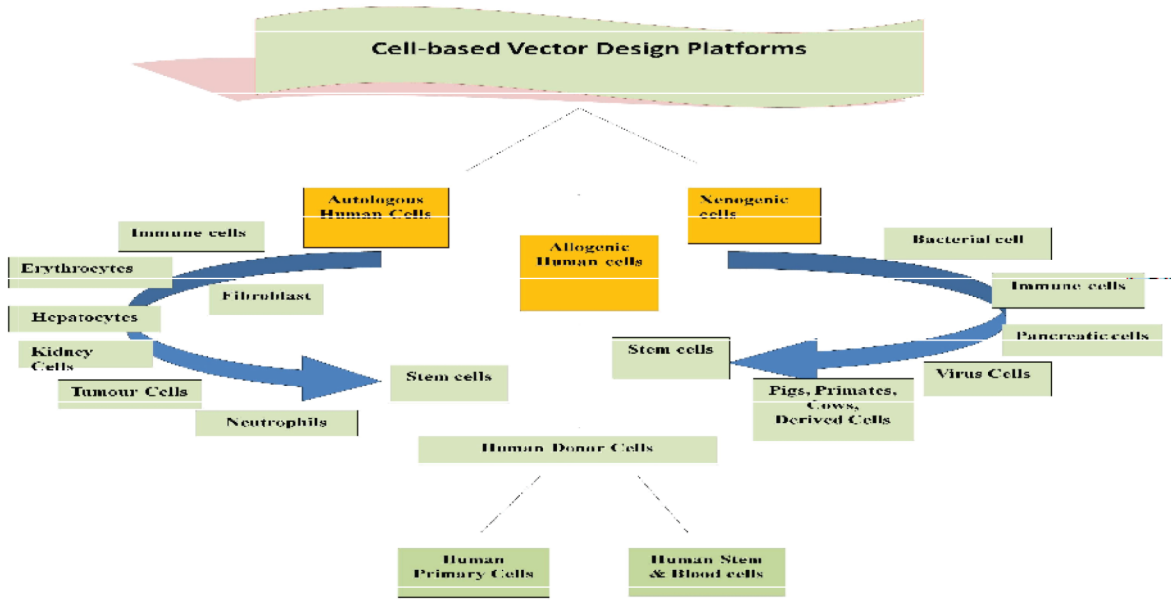

Fig. (7). Vector development and manufacturing strategies.

ment and validation of a suitable potency assay should be started in clinical trial phase and during product development phase, release and shelf life specifications for potency, ought to be determined. Biological activity assays should be related to their clinical response based on the intended effect. Effectively, there are two types of potency assays:

(1) *In vitro* assays using cell systems

(2) *In vivo* assays using animal models.

Key cellular functions like viability, self-renewal, differentiation and death are the critical characteristic of sustainability, quality and function of the cell based remedial products which must be monitored during production and at release. This can be achieved through methods like assessment of flow cytometric immune fluorescent analysis, gene expression profiles by microarrays, PCR, cell cloning *etc*. *In vivo* assays for potency may be conducted when experimental animal models are available. Markers for purity and markers for potency should maintain their identity in the assay.

3.5. Tumorigenicity

The tumorigenicity of cell based medicinal products (CBMP) differs from the classical pharmaceutical formulation as the alteration arises in the cellular component of the product (*e.g*. chromosomal instability). The cellular components should be assessed for their tumorigenic prospective by analysing *e.g*. their proliferative capacity, dependence on the exogenous stimuli, response to apoptosis stimuli and genomic modification. Testing of chromosomal integrity and tumourigenicity of cells derived from a cell culture/cell banking system will be required.

3.6. Release Criteria

For proper quality control, the active substance and/or the final product should be subjected to release testing. The release specifications of the active substance and the finished product selected should be product-specific and defined by the manufacturer. In the case of autologous products complete release testing is not required before the product is administered to the recipient due to time restrictions but retention samples should be stored for future analysis. The release of the product should be justified by the validation of the cell manipulation process and the in-process controls.

3.7. Stability Testing

A valid shelf life (after opening from the transport container) should be allotted to the CBMP and storage conditions including temperature range should be defined.

A shelf life for the cells under specified storage conditions shall be determined for the following materials:

i) All intermediates subject to storage if applicable, ii) Components of the combined, iii) The active substance, iv) The finished product. Transportation and storage conditions should be sup-

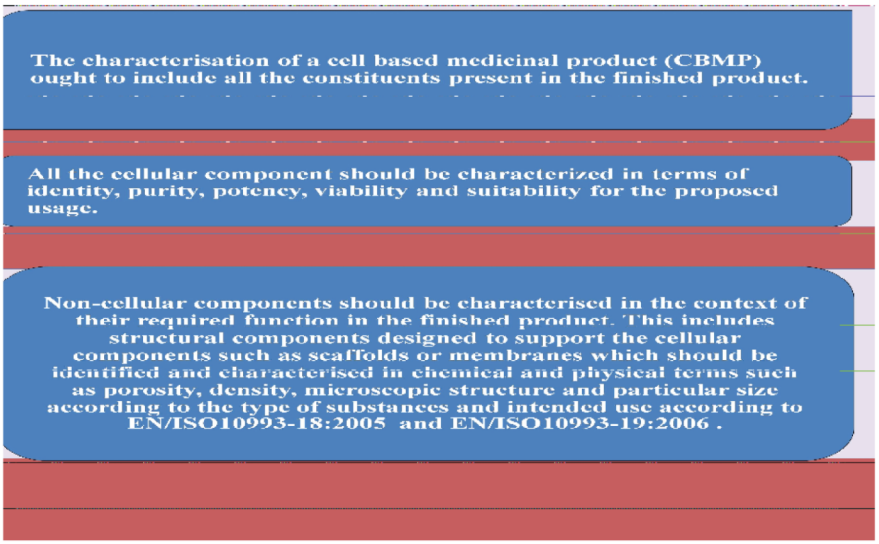

Fig. (8). Pharmaceutical requirement of cell-based product.

ported by experimental data with regard to the maintenance of cell integrity and product stability during the defined period of validity. If relevant, appropriate methods for freezing and thawing should be documented. Due to the intricate character of the active substance of a CBMP, requirements for stability should be defined on an independent basis (Fig. **8**).

4. REGULATORY REQUIREMENT OF CELL-BASED PRODUCT

For safety, quality and efficacy of cell-based product regulatory issues must be considered during preparation of a cell- and tissue-based therapy for commercial and clinical use. Safety testing includes assays for endotoxin, fungal, mycoplasma, potential microbial and viral contamination, karyotype testing and improvement for the requisite cell population. After establishment of safety, the product must get ahead of *in vitro* functional assays to measure clinical effectiveness.

4.1. Legislation on Cytotherapy in European Countries(EC)

Legislations are based on the directives for setting standards of quality and safety for human tissues and cells with respect to donation, preservation, procurement, processing, testing, storage and distribution [60].

- Directive 2001/20/EC stresses that clinical trials are compulsory for cytotherapy products and describes special requirements for sanction of such trials.
- Directive 2004/23/EC lays standards of safety and quality for the procurement, donation and testing of all human tissues and cells proposed for human application, and of prepared products derived from human cells and tissues proposed for human applications, so as to make sure high levels of human health protection.
- Directive 2006/17/EC applies Directive 2004/23/EC of Council and European Parliament referring to technical requirements meant for the donation, procurement and testing of human tissues and cells.
- Directive 2006/86/EC implements Directive 2004/23/EC of Council and European Parliament referring to, notification of serious adverse reactions, traceability requirements and events, definite technical requirements for the preservation, coding, processing, distribution, storage of human tissues and cells.
- Directive 2009/120/EC amends Directive 2001/83/EC of Council and European Parliament on the Community code which relates to medicinal products for human use with respect to advanced therapy.
- EU Regulation 1394/2007 on advanced therapy medicinal products (ATMPs) amends the Directive 2001/83/EC and Regulation (EC) No 726/2004. The EU Regulation on ATMPs is effective since December 2008 and is applicable and binding in all Member States of the Council

and European Parliament. ATMPs include gene therapy medicinal products and somatic cytotherapy products.

4.2. Regulatory Framework in the U.S. [61]

In the US, Centre for Biologics Evaluation and Research (CBER) regulates cytotherapy products, human gene therapy products, and certain devices related to cell and gene therapy. The Code of Federal Regulations unifies and codes cytotherapy products in the following sections:

- Investigational New Drug (IND) regulations (21 CFR 312),
- Biologics regulations (21 CFR 600)
- cGMP (21 CFR 211).

US federal regulations on cellular therapy are classified into two sections of the Public Health Service Act (PHSA), referred to as 351 products and 361 products. Traditional bone marrow progenitor cells, blood and other tissues for transplantation fall in the definition of 361 products.

The cell-based therapeutics which does not require FDA approval "is, minimally manipulated, labelled or advertised for homologous use only, and is not pooled with a drug or device". In contrast, treated autologous cells meet definition of somatic cytotherapy products for structural use and require exemption from filing IND or the FDA license approval. "Guidance for Industry: Regulation of HCT/Ps – Small Entity Compliance Guide" was released in 2007 and in 2009, the "Guidance for Industry on Current Good Tissue Practice (c GTP) and Additional Requirements for Manufacturers of HCT/Ps" was released. Clinical studies employing mesenchymal stem cells (MSCs) bring about the IND mechanism. Accordingly, it necessitates a comprehensive study protocol, wherein the investigators make an IND application describing the clinical plan as well as the testing and preparation of the therapeutic cell product [61, 62].

4.3. Regulatory Framework in India

The "Ethical Guidelines for Biomedical Research on Human Subjects" released by Indian Council of Medical Research (ICMR) in 2006 provides for the requirements of carrying out stem cell research and therapy under Section VII [63]. The guidelines are divided into three areas, permissible, restrictive and prohibited areas for research on stem cells. For conducting cytotherapy research following ICMR Guidelines for Biomedical Research and Good clinical practices (GCP) guidelines of the Government of India, an earlier sanction of Institutional Committee for Stem Cell Research and Therapy (IC-SCRT), Institutional Ethics Committee (IEC) and Drug Controller General of India (DCGI) is required under the permissible category.

The constrained category includes cytotherapy sponsored by multinationals for stem cell products imported from abroad. Earlier approval of national apex committee for stem cell research through DCGI, IEC, IC-SCRT and the funding agencies for such collaborations is required as per the procedure or Health Ministry's Screening Committee (HMSC). Each institution constitutes an IC-SCRT as per the guidelines and affords adequate support for its functioning.

Clinical use of stem cells was not permitted until 2007. In November 2007, "Guidelines for Stem Cell Research and Therapy" were laid down by ICMR and the Department of Biotechnology (DBT) [64] which mandates the following:

- Well established efficacy and safety procedure
- Adequately defined and labelled safety and composition of product
- Detailed conditions for use and storage to be provided.

Central Drugs Standards Control Organization (CDSCO) also released a guidance document in December 2008 on submission requirements for new drug approvals for Biotechnological/ Biological products [65] along the lines of the CTD format (Table **2**).

CONCLUSION

Transplantation of live cells as therapeutic agents offers new treatment options for acute and chronic diseases. As it enables the body to regenerate damaged and lost tissues. Stem cells are now being increasingly used in the treatment of myocardial infarction, prostate cancer, type I diabetes, graft versus host disease and other regenerative therapies owing to its potential of regeneration, However, the complexity of biological cells hampers the rendition of laboratory-scale experiments into industrial processes for cost-effective, reliable preparation of cell-based therapies.

Table 2. Regulatory framework of EU, US and India.

European Union	United States of America	India
European Medicines Agency (EMA) has 7 scientific committees and working parties out of which CHMP and CAT regulates cytotherapy products. • Committee for Medicinal Products for Human Use (CHMP) • Pharmacovigilance Risk Assessment Committee (PRAC) • Committee for Medicinal Products for Veterinary Use (CVMP) • Committee for Orphan Medicinal Products (COMP) • Committee on Herbal Medicinal Products (HMPC) • Committee for Advanced Therapies (CAT) • Paediatric Committee (PDCO) **The Committee for Medicinal Products for Human Use (CHMP)** • provides scientific advice • prepares scientific guidelines • cooperates with international partners **Committee for Advanced Therapies (CAT)** • participates in certifying quality and non-clinical data • participates in providing scientific recommendations on the classification of ATMPs; • contributes to scientific advice • advises the CHMP on any medicinal product • provides scientific expertise and advice for any Community initiative • supports the work programmes of the CHMP working parties	Food and Drug Administration, an Agency consists of following offices of which CBER regulates cytotherapy products. • FDA's Office of the Commissioner • Office of Operations Organization • Office of Policy, Planning, Legislation, and Analysis Organization • FDA's Office of Medical Products and Tobacco • Center for Biologics Evaluation and Research • Center for Devices and Radiological Health • Center for Drug Evaluation and Research • Center for Tobacco Products • Office of Foods and Veterinary Medicine • Center for Veterinary Medicine • Center for Food Safety and Applied Nutrition • Office of Global Regulatory Operations and Policy (OGROP) • National Center for Toxicological Research • Office of Regulatory Affairs (ORA) **Centre for Biologics Evaluation and Research (CBER)** Regulates blood, vaccines, allergenics, tissues, cytotherapy products, human gene therapy products, and certain devices related to cell and gene therapy. • It ensures that biological products are safe and effective and available to those who need them. • It provides the public with information to promote the safe and appropriate use of biological products.	Department of Health Research, Ministry of Health and Family Welfare, Govt. of India oversees the activities in the field of stem cell research in India. Two levels of monitoring exists for the same **National level (NAC-SCRT)** Focuses primarily on policy. Consists of independent body of experts signifying diverse areas of biomedical research, concerned government agencies and other stakeholders. a) General oversight of the field of stem cell research and therapy in India and formulation of policy related to it b) Review of specific controversial or ethically sensitive research and proposals for therapeutic use of stem cells / differentiated derivatives. ii) Institutional level(IC-SCR) A self-regulatory system. Members with specific expertise as per guidelines. a) It is mandatory for them to register with NAC-SCRT. b) Submit periodic report on their scientific activities for effective functioning.

The variation found in cases of intrapopulation heterogeneity, depends and varies further on manufacturing conditions. Further characterization techniques of cytotherapy products need to be strengthened to appreciate the existence, phenotype, and impact of cellular subpopulations for cytotherapy. Autologous products are now an approved treatment for cancer as per PMDA Japan. Provenge, a prostate cancer vaccine induces immunity against expressed PAP antigen. It is a therapeutic vaccine administered after cancer has been diagnosed. Prochymal, used against aGvHD improves the overall survival probability with no reported adverse effects.

Preservation of cell lines is desirable from many perspectives, if a graft fails, preservation allows multiple episodes of treatment for the patient with the same batch of cells. The processes utilized in the preservation of cell lines compromise the immunosuppressive properties for the

reason that heat-shock results due to cryopreservation. Cytotherapy products face several manufacturing challenges as acceptance criteria for the amounts of contaminating cells should be set. Markers for purity and potency should maintain their identity during assay. Tumorigenic potential of cellular components should be assayed. The release criteria are validated by in process control and cell manipulation. A regulatory requirement for valid shelf life (after opening from the transport container) should be allotted to the CBMP and storage conditions including temperature range should be defined by the respective regulatory authorities of various countries.

As of the current scenario, there is much left to be done in the clinics and laboratories to comprehend the use of various cells for cytotherapy. Stem cell research is progressing rapidly towards identifying birth defects, and studying normal growth. Research on cytotherapy still raises scientific questions as rapidly as it generates new discoveries. Rapid advancements are required to answer the growing questions and making the discoveries translational. As far as the innovations are concerned, they should be more and more realistic and should focus on smooth transition from bench to bedside.

ETHICAL STATEMENT

This article does not contain any studies with human or animal subjects performed by any of the authors.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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