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Advances and Challenges in Stem Cell Culture

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Abstract: In recent years, stem cell therapy has become a very promising and complex scientific research topic. In the body, stem cells have the capacity to differentiate into a wide variety of cell types. They are capable to regenerate body system. Adult stem cells and embryonic stem cells are the two primary categories of stem cells. Through cell-replacement therapies, stem cell-based therapies can give the opportunity to replace lost, damaged, or old cells in order to regenerate or repair tissues and organs. Additionally, using genetically altered stem cells as delivery systems holds enormous potential for treating inherited genetic flaws and delivering therapeutic chemicals to specifically injured tissues and organs. The ensuing integration of science and medicine made possible by more research will enable the therapeutic application of ES cell technology. In the end, such a program might present a remarkable new weapon in the fight against human sickness.

Keywords: Stem cells; Embryonic cells, future medicine, ES cell technology.

REVIEW PAPER

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INTRODUCTION

In the body, stem cells have the capacity to differentiate into a wide variety of cell types. They are capable to regenerate body system. Adult stem cells and embryonic stem cells are the two primary categories of stem cells. In recent years, stem cell therapy has become a very promising and complex scientific research topic. High hopes have been raised by the development of medical techniques. This article provides a summary of several stem cell discoveries and potential treatments based on these cells. Stem cell genesis is followed by controlled stem cell derivation and culture in the lab. In order to assess the properties of the stem cells under consideration, quality control and teratoma development tests are essential. The ideal environment for controlled differentiation must be created, which depends on the usage of culture medium and derivation processes. Among the many different stem cell applications, the usage of graphene scaffolds and the potential of extracellular vesicle-based therapies require special attention due to their versatility (Zakrzewski *et al*., 2019).

Mesenchymal Stem Cells

Adult stem cells that can develop into numerous cell types are called mesenchymal stem cells (MSCs), which have been obtained from several sources. These sources can come from the bone marrow, fat (adipose tissue), umbilical cord tissue (Wharton's jelly), or amniotic fluid (the fluid surrounding a fetus) in humans (Ding *et al*., 2011).

Epidermal stem cell

Adult skin homeostasis and hair regeneration are maintained by stem cells (SCs) found in the epidermis and hair follicle, but they also take a role in the epidermis' ability to heal after damage (Stern & Bickenbach, 2007).

Advances in stem cell

Recent advances in fundamental and clinical research on adult, UCB, fetal, amniotic, and embryonic stem cells have opened up a wide range of options for their potential therapeutic use in human cancer and regenerative medicine. Through cell-replacement therapies, stem cell-based therapies can give the opportunity to replace lost, damaged, or old cells in order to regenerate or repair tissues and organs. Additionally, using genetically altered stem cells as delivery systems holds enormous potential for treating inherited genetic flaws and delivering therapeutic chemicals to specifically injured tissues and organs. Stem cells and/or their further differentiated progeny

are cells with immense therapeutic promise for treating and even curing a variety of hereditary and degenerative problems in the human body. However, these pathological disorders are still incurable with current clinical therapy options (Mimeault *et al*., 2007).

Stem cell use in medicine

According to the available data, ES cells have immense promise for use in cell replacement treatment and regenerative medicine, even though they are not yet ready to make their clinical debut. According to Wu et al. all of the fetus' tissues might theoretically be created by ES cells, and experimental research has already largely realized this promise. A significant accomplishment of contemporary science is the differentiation of ES cells into neurons, hepatocytes, cardiomyocytes, insulin-producing cell clusters, and hematopoietic precursors. The ensuing integration of science and medicine made possible by more research will enable the therapeutic application of ES cell technology. In the end, such a program might present a remarkable new weapon in the fight against human sickness.

Stem cells as a target for pharmacological testing

An abnormal immunological response and a persistent lack of remyelination in the central nervous system are two features of multiple sclerosis. Current therapies only focus on the immune system, but remyelination through the production of new oligodendrocytes is necessary to prevent brain degeneration and subsequent impairment. The primary source of myelinating oligodendrocytes, oligodendrocyte progenitor cells are stem cells in the central nervous system. These cells are numerous in demyelinated areas of multiple sclerosis patients but do not differentiate, making them a potential target for pharmaceutical intervention. the search for medicinal substances capable of promoting myelination in endogenous oligodendrocyte progenitor cells. We tested a collection of bioactive small compounds on oligodendrocyte progenitor cells generated from mouse pluripotent epiblast stem cells, and the results demonstrate that seven of the medicines work at nanomolar levels to improve the in vitro maturation of oligodendrocytes. Miconazole and clobetasol are two medications that can effectively encourage premature myelination in organotypic cerebellar slice cultures and in vivo in young postnatal mouse pups. In a lysolecithin-induced animal model of focal demyelination, systemic administration of each of the two medications greatly boosts remyelination and increases the number of new oligodendrocytes. An experimental autoimmune encephalomyelitis mouse model of chronic progressive multiple sclerosis exhibits a dramatic reversal of disease severity with the administration of each of the two medicines at the height of the disease. In contrast to clobetasol, which is a potent immunosuppressant as well as a remyelinating agent, immune response experiments demonstrate that miconazole acts directly as a remyelinating medication with no effect on the immune system. According to mechanistic research, miconazole and clobetasol act on oligodendrocyte progenitor cells through signaling through the mitogen-activated protein kinase and the glucocorticoid receptor, respectively. Additionally, both medications improve human oligodendrocyte in vitro differentiation from human oligodendrocyte progenitor cells. Overall, our findings support the study of derivatives of miconazole and clobetasol with structural modifications to improve remyelination in patients (Najm *et al*., 2015).

Stem cells as an alternative for arthroplasty

Embryonic stem cells may be used in cutting-edge immunomodulatory procedures such hematopoietic chimerism techniques intended to promote tolerance to donor organ allografts. Sadly, cancer and random differentiation continue to impede the development of embryonic stem cell therapies, and it is still unclear how the immune system will react to a tissue graft made from these cells. This overview highlights the achievements to date and the upcoming challenges still to be resolved in embryonic stem cell research for transplantation. Despite the fact that embryonic stem cells are still years away from making their clinical debut, ongoing research gives hope that they may eventually play a significant role in cell replacement treatment and regenerative medicine (Wu *et al*., 2007b).

Therapy for incurable neurodegenerative diseases

Research on stem cells has grown significantly in recent years as a method for creating new therapeutics for neurodegenerative illnesses that are incurable. Although stem cell transplantation has shown promise in a number of animal models, it is still unclear whether fundamental mechanisms of healing are involved. In addition to the replacement of lost cells, a number of mechanisms, including cell fusion, neurotrophic factor release, endogenous stem cell proliferation, and Tran's differentiation, may account for favourable therapeutic outcomes. To optimise the possibility for efficient treatments, the biological problem needs to be defined. Amyotrophic Lateral Sclerosis (ALS) has recently been highlighted as an ideal candidate disease for the development of stem cell therapy in humans due to the lack of any viable pharmaceutical treatments and early findings in both experimental and clinical settings. On patients, preliminary stem transplantation experiments have previously been carried out. In particular, the review debates the subject of Tran's differentiation, endogenous neural stem cells, and variables influencing stem cell fate. It also examines pertinent topics surrounding the application of stem cell research to ALS but also generally to other neurodegenerative disorders in 2004 (Silani & Corbo).

Stem cell use in dentistry

The incapacity of most tissues and organs to heal and regenerate after damage is a challenge that has to be tackled in light of exceptional advancements in the prevention, diagnosis, and treatment of human diseases. In an effort to make significant medical advancements, stem cell research is being undertaken. Scientists are working to develop treatments that repair or swap out damaged cells with new ones made from stem cells, giving hope to those suffering from a variety of illnesses. Dentists today have to deal with the regeneration of injured dentin, pulp, bone, and periodontal tissue. The ability to restore and regenerate teeth and periodontal structure lies in the stem cells that are found in dental pulp, periodontal ligament, and alveolar bone marrow. These stem cells can be extracted from dental pulp, periodontal ligament, and/or alveolar bone marrow, expanded, embedded in a suitable scaffold, and then re-implanted into a defect to restore bone and tooth tissues. These cells can be employed to repair bone abnormalities and have the capacity to rebuild cementum, periodontal ligament, and dentin. Consideration must be given to the type of surgical method to be employed, the type of scaffold, the source of cells, the type of in vitro culturing, and other factors. The working dental surgeon is an essential part of the interdisciplinary project. Knowing the enormous potential of using stem cells in a clinical setting and having a thorough comprehension of the issues involved are necessary for performing this function in the best possible way (Mitsiadis and others, 2015).

Grand challenges in stem cell treatments

Manufacturing issues

Products used in allogeneic cell therapy, such as therapeutic cells produced from pluripotent stem cells (PSCs), have the extraordinary potential to treat a wide range of diseases and a sizable population of patients worldwide. The production of PSCs in sufficient quantities to satisfy commercial needs is fraught with difficulties, though. In addition to discussing the difficulties in developing a procedure for producing PSCs in a bioreactor, this manuscript also introduces a scalable bioreactor technology that may provide a way to bypass the bottleneck in the creation of high-quality therapeutic cells from PSCs on a wide scale (Lee *et al*., 2020).

Genetic instability

The use of induced pluripotent stem cells (iPSC) is crucial in regenerative medicine. Nevertheless, it is becoming more and more obvious that the reprogramming procedure, involving retroviral transduction with strong oncogenes like c-Myc and prolonged cultivation, may result in genomic instability. IPS cell cultures may become dominated by genetically modified cells as they proliferate. The goal of this study is to thoroughly synthesis the present understanding of the genetic instability of embryonic and iPSCs, with a focus on cytogenetic changes, and to correlate these findings with what is known about cancer (Ben-David *et al*., 2010).

Stem cell culture condition

Today, three different stem cell types are typically expanded in culture. Induced pluripotent stem cells, adult stem cells, and embryonic stem cells (ES) (iPS). The inner cell mass of the blastocyst is where pluripotent stem cells, or embryonic stem cells, are produced. They have an endless capacity for self-renewal under the right circumstances, yet passage into culture may cause them to exhibit particular karyotypic anomalies. In developed tissues, multipotent cells called tissue-specific stem cells can be discovered. These adult stem cells may not self-renew fully in culture. Current stem cell culture conditions need to be improved, according to the restricted capacity of adult stem cells for self-renewal in culture and the cytogenetic alterations seen in highly passaged ES cells. This is a very important aspect because, as will be explained below, cultural factors can have a big impact on stem cell fate. Induced pluripotent stem (iPS) cells, the third form of stem cell, are also being grown at the moment. These cells are somatic cells that have undergone pluripotent stem cell reprogramming with the addition of a few genes, such as the SOKM (Sox2, Oct4, Klf4, and c-Myc) or SOLN (Sox2, Oct4, Lin28, Nanog) combinations. It might be claimed that because of their diverse ancestries, it is impossible to define an ideal stem cell culture regime that would apply to all types of cultivated stem cells. All stem cell types, however, now face roughly the same difficulties with respect to the circumstances of stem cell culture. Adult stem cells are found in the three-dimensional stem cell niche, which is a highly specialised milieu in vivo. For technological reasons or because the processes that control the stem cell destiny in vivo are still poorly understood, it is not possible to replicate this complex and dynamic microenvironment in culture. The medium, the atmosphere, the substrate, and the interactions between the cells that make up the environment that cultivated cells are exposed to in vitro. Every one of these parts is involved in an intricate web of signalling pathways that determines the stem cell fate. In the sentences that follow, this will be covered. The period in which cell culture conditions were established empirically is over. An area of study that is rapidly expanding and has enormous implications for our understanding of stem cell biology and regenerative medicine is that of the impacts of medium, atmosphere, substrate, and cell-cell interactions (Van Der Sanden *et al*., 2010).

Pharmacological issue

Worldwide, the prevalence of autism spectrum disorders (ASD) has substantially increased during the past ten years. The key co-occurring symptoms of ASD include the existence of restricted and repetitive behaviours, as well as communication and social interaction deficits, which are not well treated by the current crop of medications. On the other hand, transplanting hematopoietic and mesenchymal stem

cells into ASD children has showed promise in lowering inflammation and alleviating some ASD symptoms by encouraging the recruitment, proliferation, and differentiation of tissue-residing native stem cells. ASD has also been linked to a number of comorbid conditions, including immunological dysregulation, gastrointestinal problems, and dysbiosis of the gut microbiota. Non-pharmacological strategies, like dietary supplements with specific vitamins, omega-3 polyunsaturated fatty acids, probiotics, some phytochemicals (like luteolin and sulforaphane), or general dietary interventions (like gluten- and casein-free diets), have been considered for the reduction of such comorbidities and the management of ASD. Here, interventional studies reporting stem cell-based therapies and pharmacological and non-pharmacological treatments for children and adolescents with ASD are reviewed (Pistollato and others, 2020).

Stem cell distribution after transplant

Adult bone marrow, fat, and a number of foetal tissues can all be used to make mesenchymal stem cells (MSCs). MSCs are capable of being multiplied in vitro and can differentiate into a variety of mesenchymal tissues, including bone, cartilage, and fat. They are immune system-escapers in vitro, which may make them suitable for cellular treatment in an allogeneic environment. Additionally, when combined with hematopoietic stem cells, they may lessen graft-versus-host disease (GVHD), prolong skin allograft survival, suppress T-cell proliferation in mixed lymphocyte cultures, and have immunomodulatory effects. MSCs use a soluble factor to cause their immunosuppressive action. Contradictory findings exist; this may be because of variations in the cells and systems investigated, but a number of candidates and mechanisms have also been put forth. The fact that it has been challenging to locate and separate MSCs following in vivo transplantation has been a significant issue. However, in patients who have received both autologous and allogeneic grafts, MSCs appear to improve hematopoietic engraftment. They have recently been discovered to reverse grade IV acute GVHD of the liver and intestines. But no tolerance was brought on. Controlled studies are necessary. MSCs can therefore be employed in allogeneic stem cell transplantation for hematopoiesis augmentation, GVHD prevention, and the treatment of severe acute GVHD. In autoimmune inflammatory bowel diseases and organ transplant rejection, where immunomodulation and tissue healing are required, they may also prove useful (Le Blanc & Ringdén, 2005).

Challenges in a developing country

This research identifies key distinctions across global value chains (GVCs) supplying the European clothing markets through an examination of clothing import trends and sourcing methods of significant apparel retailers in the UK, France, and Scandinavia. It emphasises the difficulties that developing nation suppliers have when trying to join the supply chains of UK retailers and ties these difficulties to corporate financialization in the country. The growth of GVCs contradicts traditional "industrial upgrading" paradigms and the role of the apparel sector as a stepping stone in the industrialization of underdeveloped countries, albeit suppliers' entry and industrial upgrading remain simpler in mainland European sourcing networks (Palpacuer *et al*., 2005).

Stem cell obstacles in the future

The safety and ethics of cutting-edge scientific and medical developments must always be properly monitored. It should not be treated differently because stem cell therapy already has a significant impact on many facets of life. Stem cell research is currently faced with a number of difficulties. The most crucial one is the first one, which focuses on properly comprehending how stem cells work in animal models first. You cannot skip this step. Fear of the unknown is the biggest obstacle to achieving the procedure's widespread, global acceptability.

Modern advances in science and medicine must always be adequately monitored for safety and ethics. Because stem cell therapy already significantly affects many aspects of life, it shouldn't be handled any differently. Currently, there are many challenges facing stem cell research. The first one, which concentrates on first fully understanding how stem cells function in animal models, is the most important one. This step must be completed. The main impediment to the procedure's vast, universal acceptance is fear of the unknown.

Modern advances in science and medicine must always be adequately monitored for safety and ethics. It must. An additional difficulty is identifying and correctly isolating stem cells from a patient's tissues. Successful stem cell transplantation is significantly hampered by immunological rejection. It is possible for the immune system to mistake transplanted cells for foreign substances when using specific stem cell kinds and techniques, leading to transplant or cell rejection.

Implementing a self-destruct option in the event that stem cells become harmful is one of the concepts that can make stem cells "failsafe." Stem cell research and flexibility could result in lower therapy costs for patients with diseases that are currently incurable. Instead of undergoing extremely costly pharmacological treatment while confronting specific organ failure, the patient would be able to use stem cell therapy. In the event of a successful procedure, the patient would avoid long-term pharmaceutical therapy and its unavoidable negative effects.

Despite these formidable obstacles, the area of stem cell research is making tremendous strides every day. Several diseases and ailments can already be treated with stem cell treatment. Their influence on medical advancements in the future seems to be substantial (Zakrzewski and others, 2019).

Why stem cells are difficult to culture

For some uses, stem cells might be challenging to cultivate due to various issues they present. Successful culture requires a biocompatible culture media, which is typically achieved by coating the vessel with cells of a closely related type. However, a "Feeder layer" is necessary since stem cells have an infinite capacity for self-renewal. A layer of cells that have been given growth-inhibiting treatment is known as a feeder layer. The target cells must receive nutrition, which is necessary. Cells from mouse embryos were traditionally used to create this feeder layer. As the feeder layer could transmit viruses to the cell culture, this alone can be problematic. Despite the fact that its influence has been reduced by modern procedures, it is yet a crucial safety issue. It can take several tries to attach target cells to the culture vessel and is also challenging to create an embryonic stem cell line from scratch. Our infographic discusses the consequences of improper stem cell culture, including the high expenses, delays, and reputational harm that can result.

CONCLUSION

The use of stem cells (SCs) in cell therapy, tissue engineering, and regenerative medicine, as well as in pharmacological and biotechnological applications, holds enormous potential. Depending on the source of their isolation, they can self-renew and differentiate into different cell types with specific functions. However, using SCs for medical purposes necessitates a large number of high-quality cells. Due to this, SCs must be expanded widely before differentiating effectively and uniformly into functional derivatives. The use of plastic culture plates with xenogenic medium in two-dimensional (2-D) cultures is a traditional strategy for cell maintenance and expansion. When cells are sent around for an extended period of time using these techniques, they tend to lose their ability to differentiate and clone. In order to imitate the in vivo environment, recent advancements in SC expansion techniques have placed a strong emphasis on three-dimensional (3-D) cell development. Inculcating SCs in 2-D and 3-D environments with spheroids, biomaterials, and bioreactors has recently undergone significant developments, which are all compiled in this review. The discussion also includes potential obstacles to achieving a billion-fold growth of cells.

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