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APPLICATIONS OF HUMAN PLACENTAL CHORION-INDUCED PLURIPOTENT CELLS FOR TISSUE ENGINEERING

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ABSTRACT

Stem cells, which have an enormous capability for self-renewal and can transform into many diverse cell types in the body early in life and during growth, have been heavily researched in recent years. Stem cells are specialized cells that can regenerate and repair damaged or diseased organs in humans. They are employed in regenerative medicine and tissue engineering. Thus, beneficial outcomes have been observed in the treatment of many disorders and faulty tissues. Mesenchymal stem/stromal cell lines (abbreviated as MSCs) isolated from fetal and adult tissues are of considerable interest for use in tissue engineering and cell therapeutics, thanks to their ability to migrate, regenerate, and repair injured sites. In this review study, induced pluripotent stem cells were derived from human placental chorion to advance tissue engineering technologies and investigate therapeutic approaches to various disorders.

Keywords: Stem cell, Human Placental Chorion, Tissue Engineering, iPSC, CMSC

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DOKU MÜHENDİSLİĞİ İÇİN İNSAN PLASENTAL KORYONUNDAN GELİŞTİRİLEN PLURİPOTENT HÜCRELERİN UYGULAMALARI

ÖZET

Yaşamın erken dönemlerinde ve büyüme sırasında vücutta çok çeşitli hücre tiplerine dönüşebilen ve kendini yenileme konusunda muazzam bir yeteneğe sahip olan kök hücreler son yıllarda yoğun bir şekilde araştırılmıştır. Kök hücreler, insanlarda hasarlı veya hastalıklı organları yenileyebilen ve onarabilen özel hücrelerdir. Kök hücreler rejeneratif tıp ve doku mühendisliği için kullanılır. Bu nedenle, birçok rahatsızlığın ve hatalı dokunun tedavisinde faydalı sonuçlar gözlemlenir. Fetal ve yetişkin dokulardan izole edilen mezenkimal kök/stromal hücre hatları (kısaca MSC'ler), göç etme, yenilenme ve yaralı bölgeleri onarma yetenekleri sayesinde doku mühendisliği ve hücre terapötiklerinde kullanım için önemli ilgi görmektedir. Bu inceleme çalışmasında, doku mühendisliği teknolojilerini ilerletmek ve çeşitli rahatsızlıklara yönelik terapötik yaklaşımları araştırmak için insan plasenta koryonundan indüklenmiş pluripotent hücreler türetilmiştir.

Anahtar Kelimeler: Kök hücre, İnsan Plasenta Koryonu, Doku Mühendisliği, iPSC, CMSC

1. INTRODUCTION

Stem cells are sources of cells in multicellular organisms that are partially differentiated or undifferentiated, self-replicating, able to survive for long periods, and live in microenvironments called niches, specialized structures that contributes to the continued existence of stem cells [1]. Under normal conditions in tissues, these cells maintain their population and provide tissues with new functional properties. Stem cells have an extraordinary potential for self-renewal. During early embryonic development and growth, they can differentiate into numerous distinctive cell types within the body. These cells divide symmetrically and asymmetrically in an unlimited manner, as there is no Hayflick limit. As a result of serial division, clonogenicity leads to the generation of either stem cells or committed/differentiating cells. In addition, although it is accepted that there is no Hayflick limit in stem cells, these cells cannot be passaged for long periods in the laboratory because they begin to differentiate as the passage number increases. This differentiation has led to the

classification of stem cells into to five categories: pluripotent, totipotent, multipotent, unipotent, and oligopotent stem cells [2]. Totipotent cells are embryonic stem cells capable of producing all parts of the human body, including placental, fetal, and embryonic tissues as well as extraembryonic tissues. A human zygote is created when an egg and sperm combine. During the morula stage of embryonic development, the newly formed zygote's cells divide and continue to be totipotent. The cleavage phase of this embryonic stage lasts from one to six days. A grape cluster shape with 8 cells is visible at the morula stage. A blastocyst, with a distinctly characterized inner cell mass, a fluid-filled blastocoele, and a layer of trophoblasts is then formed from the morula. Cells in the blastocyst state lose their totipotency. Clinically, the best evidence of the differentiation of totipotent cells into different lineages is seen in the evolution of dichorionic-diamniotic monozygotic twins [3, 4].

Pluripotent stem cells, a type of embryonic stem cells, are stem cells that cannot differentiate into extraembryonic tissues but can differentiate into any of the endoderm, ectoderm, and mesoderm germ layers. Since they can produce cell lines originating from any of the three germ layers, they can give rise to all fetuses. Yamanaka and Takahashi (2006) were the first to demonstrate how adult somatic cells, such as fibroblastas, can be made pluripotent. In their study, the scientists were able to change the behavior of mouse skin fibroblasts to resemble that of embryonic stem cells [5]. Takahashi and Yamanaka conducted a similar investigation on human cells in 2007 [6]. It was long believed that a specialized or mature cell could not revert to a young state. Yamanaka et al. 24 potential genes involved in reprogramming somatic cells toward pluripotency. Many of the candidates' retroviral transduction did not alter the cell in any way. Nevertheless, some colonies diverged from their fibroblast characteristics and became similar to embryonic stem cells. Among the 24 genes, only four - *KLF4, SOX2, OCT3/4, and c-MYC* - can induce ectopic expression and reprogram the cell to be similar to resemble embryonic stem cells, both physically and functionally. The placenta, a unique and temporary fetomaternal organ, has gained attention as a significant and valuable source of stem cells. Immature hematopoietic progenitors and hematopoietic stem cells can be detected in the placenta, a considerably vascularized hematopoietic tissue [7].

The placenta is a mammalian organ that connects the fetus to the uterine wall, while the umbilical cord connects the fetus to the placenta. After fertilization, the inner cell mass of the blastocyst differentiates into hypoblasts and epiblasts. The hypoblast produces the placenta's

umbilical cord and vessels, whereas the epiblast produces the placenta's amniotic membrane or inner layer, as well as the fetus. The amniotic membrane, also known as the amnion, is composed of mesenchymal-like cells that form an epithelial layer that surrounding the amniotic cavity. The outer layer of blastocyst, or trophectoderm, produces trophoblasts, which form the chorionic membrane of the uterine wall or the outer layer placenta [8].

The chorion is the outher layer of the human extraembryonic fetal membrane that connects the growing fetus to the maternal tissues during pregnancy. The chorionic membrane is in proximity to the decidua and is seperated from the amniotic membrane by a layer of spongy collagen fiber. During the first trimester of pregnancy, the chorion rapidly proliferates, forming multiple chorionic villi that eventually grow into the placenta. Because the formation of additional embryonic tissues occurs shortly after implantation, the cells remain immature as the entire embryo develops. Thus, the placenta and umbilical cord, which were previously discarded at birth, are now recognized as a valuable source of stem cell-like plasticity [9].

Mesenchymal stromal/stem cells (MSCs), isolated from adult tissues and fetal tissues like chorion, are of considerable interest for application in cell therapeutics and tissue engineering due to their ability to migrate, regenerate, and repair injured sites. Mesenchymal stromal cells are obtained from the trophoblastic and chorionic mesenchymal regions of the chorion. For this reason, they are divided into two subclasses: chorionic villous/placental mesenchymal stem/stromal cells (PMSCs) and chorionic mesenchymal stem/stromal cells (C-MSCs). To separate MSCs from the chorionic membrane (CMSC), the tissue is initially treated with dispase to remove the trophoblastic layer and then digested with a collagenase/DNase or collagenase mixture [10]. According to Portmann-Lanz (2006), chorion-derived mesenchymal cells display superior differentiation capacity compared to amnion-derived cells in terms of chondrogenic, osteogenic, myogenic, and neurogenic processes. The chorion is derived from the trophoblast, while the amnion is obtained from the embryoblast, the internal layer of the blastocyst [11].

Chorionic mesenchymal stromal cells (CMSCs) appear from the first trimester of pregnancy to late gestation. Human chorionic cells can be effortlessly produced and cultured from a microscopic sample in the primary culture. CMSCs can differentiate into osteoblasts, myofibroblasts, adipocytes, nerve-like cells, and hepatocytes. They can also transform into cardiomyocytes, hepatocytes, or endothelial cells. CMSCs are specialized as placental MSCs,

despite enhanced expression and their variability of pluripotent markers, according to criteria defined by the International Workshop on Placenta-Derived Stem Cells. These cells can be successfully reprogrammed. They have enhanced immunomodulatory capabilities and epigenetic memory resembling that of pluripotent cells, which may be valuable for allogeneic approaches [12].

In regenerative medicine, stem cells are used to repair and rebuild the body's damaged tissues and organs. Regenerative medicine is one of the most advanced medical treatment that can replace or restore tissues and organs devastated by age, illness, or trauma, as well as normalize inborn malformations. Thus, promising preclinical and clinical data show that regenerative medicine is being used to treat diseases affecting a wide variety of organ systems and ligaments, including chronic and acute injuries, dermal wounds, cardiovascular diseases, cancer treatments, and more. Barriers to conventional therapies for treating organ and tissue failure and loss, such as grafting of healty tissues and organs, limited donor availability, and severe immunological problems, can be overcome the use of regenerative medicine approaches. Tissue engineering is a critical component of regenerative medicine for tissue repair [13].

Tissue engineering is a scientific field that focuses on the development of scaffolds used as biomaterials that provide a 3-dimensional (3D) culture medium suitable for biological application that can replace diseased or damaged tissue in humans. Its primary purpose is to propose novel approaches for ensuring the proper functioning of bodily tissues and organs and promoting health, particularly when disorders arise within them. However, it does more than simply regenerate injured or diseased tissue; it also influences cell fate and seeks new diagnostic techniques. Tissue engineering combines biological compounds, such as growth and cell factors, with engineering techniques and synthetic materials [14]. These treatments incorporate autologous or allogeneic cells that have differentiated and are still reproducing. The scaffold is incubated in growth factor-containing conditions, which stimulate cell growth and division, and as the cell expand, replacement tissue forms. The scaffold can then be grafted into the human body and subsequently resorbed or eliminated [15].

In the 1960s and 1970s, the fields of tissue engineering and regenerative medicine focused on grafting somatic cells to the sites of a lesion to fill the crucial space between the increasing number of patients on the waiting list for organ transplants and the limited availability of donated organs. However, these efforts were largely unsuccessfully. Later, with

the development of biomaterial scaffolds, biomimetic environments that enhance cell maintenance and differentiation were produced and widely used in this field. By creating structures that resemble natural tissues, these methods provide an alternative solution. Four key elements need to be considered to create a tissue engineering plan. The cells must be able to restored the tissue's functionality. For instance, SCs and bone tissue cells must be capable of differentiating into bone cells if they are to be used for bone regenaration [15].

The culture medium should include all of the cytokines and growth factors required to encourage cell differentiation into the target cells, to promote proliferation and expansion, and to keep the desired cells alive and performing their expected functions in the tissue. The porous matrix should imitate the tissue's extracellular matrix, allowing cells to penetrate and regenerate the damaged tissue. It must possess the desired physical and chemical properties and be composed of biocompatible, bioabsorbable, and biodegradable components that promote cell growth. Finally, the bioreactor must imitate physiological conditions, providing optimal circumstances for cell proliferation and proper distribution in the scaffold [16,17].

Somatic cells, such as osteoblasts and chondrocytes, were among the earliest cell types exploited in tissue engineering applications. Adult tissue-derived stem cells, particularly MSCs, have shown promise in cell treatments and therapeutic applications due to their growth and multipotency. Cartilage, heart, bone tissue, and skin, which are candidate tissues for tissue engineering, scaffolds, and bio-artificial tissues are being investigated for use in the manufacture of bio-implanted limbs. The first successful demonstration of such a limb, a mouse leg with working muscles and arteries, was announced in 2015 [18]. Following the first developments in 3D printing, which Charles W. Hull referred to as "stereolithography" at the beginning of the 1980s, new techniques and methods for manufacturing 3D objects have emerged. These techniques are now being utilized for education, research, and even clinical practice. Initially, stereolithography was known as Photo-Doping. Resin printing, or optical production, was used to produce 3D objects by consecutively printing small layers of an ultraviolet-engineered substance. Since then, various production methods have been devised to automate the creation of individualized, machine-modeled tissue clones and organs [19].

In general, biofabrication is the process of creating bioactive chemicals, biomaterials, living cells, cell aggregates like microtissues, and hybrid cell-material structures using bioprinting or biomontage to create physiologically functioning products with structural

organization. Bioprinting is the process of using computer-aided transfer and manufacturing procedures for placing biochemicals, biological materials, and living cells to construct bioengineered structures of biological and biologically derived materials. The study reveals that using a layer-by-layer method with 3D printing for manufacturing tissue with structural control ranging from micro- to macro-scale has considerable potential [20]. The objective of 3D printing scaffold-free or scaffold-supported tissue engineering constructs is to create a biomimetic structural environment that supports host tissue integration and enables the development of new tissue (e.g., cellular infiltration, vascularization, and active remodeling). The integration of biomimetic elements into a bioprinted structure influences the adhesion, migration, growth, and functionality of both internal and external cells in a dynamic manner. Materials greatly influence the size, shape, and attachment of cells; therefore, a scaffold can regulate cell proliferation and differentiation [21].

There are several therapeutic options for stem cells. The most traditional and widely utilized multipotent stem cell therapy is hematopoietic stem cell transplantation [22]. Peripheral blood, cord blood, and bone marrow are the sources of stem cells. The patient's own cells are transplanted for specific therapeutic purposes. However, leukemia and other hematologic malignancies, such as bone marrow dysfunction are now frequently treated by allogeneic stem cell transplantation. The utilization of bone marrow transplants has greatly increased as a result of new immune-related research. Therefore, it is now possible to prevent graft-versus disease, which results in tissue incompatibility in recipients of allogeneic stem cell transplants. Therefore, cells from the patient should be utilized in new stem cell therapies, but the absence of such cells won't be a concern. Numerous scientists have tried to produce hESCs from a person's original somatic cells through nuclear transfer due to ethical concerns about using human embryos to develop pluripotent stem cells, as well as issues such as immunological rejection after transplantation. When Dolly the Sheep was cloned using nuclear transfer in 1996, this technique became more well-known [23].

These days, human disease pathophysiological pathways are studied using iPSCs as *in vitro* models. The iPSC approach has been beneficial for researching the pathophysiology of neurological diseases in humans, which is challenging to investigate due to the inaccessibility of nervous tissue. Under specific conditions, somatic cells from organs like the liver, fat, and blood have been transformed into iPSCs. Given its extraembryonic nature and ability to

reprogram induced pluripotent stem cells, the placenta—an organ that is removed at birth—has been the subject of much research. PMEDSAH, a synthetic polymer matrix, can successfully create new human iPSC lines from fetal chorionic mesenchyme cells that grow continuously under feeder-free culture conditions [24, 25].

Differentiated cell types derived from CMSC-iPSCs, can be used to test pharmaceuticals for newborns with congenital defects and to model disease. Since many pediatric diseases, such as congenital heart disease, pulmonary hypoplasia, and development brain disorders, are identified during pregnancy, these models can be used for ongoing research after the child is born. Pediatric neurological disorders such as neonatal hypoxic-ischemic encephalopathy, cerebral palsy, spina bifida, hypoplastic left heart syndrome, and fulminant liver failure, may be addressed by purified differentiated cell populations derived from CMSC-iPSCs *in vitro* [26, 27]. Another study found that 3B placental buds derived from IPSCs successfully differentiated into several trophoblast lineages, including cytotrophoblast-like, syncytiotrophoblast-like, and extravillous trophoblast-like cells, through the formation of structured layers. They were then administered to immune-deficient animals. The study yielded a success rate of 22%. This model may be useful for studying the pathophysiology and potential therapies of placental dysfunction [28]. Because they may replace animal models and have physiologic relevance, hiPSC-derived trophoblast models are a vital alternative to cell lines in placenta-on-chip systems. They can also be used to create patient-specific models. The feasibility of using trophoblasts grown from hiPSCs to produce a functioal syncytium layer in placenta-on-chip devices was demonstrated by another study. This work offers a crucial resource that may be further investigated to enhance placental barrier models in addition to insights into the impact of the microfluidic environment on hiPSC-derived trophoblast models [29].

Placenta-on-a-chip systems have been developed recently. While they present intriguing potential for placental barrier modeling, they are not suited for high-throughput research because they lack include physiologically realistic trophoblasts. This was made possible by a study that developed a placental barrier model by putting trophoblasts grown from hiPSCs into a microfluidic device. HiPSC-derived trophoblasts developed a three-dimensional structure that exhibited fusogenic and endocrine activity, placental carriers, and invasive behavior when grown in a microchannel perfused with a collagen-based matrix. These results

demonstrate that it is possible to generate a differentiated primitive syncytium from hiPSCs on a microfluidic substrate. This discovery provides a substantial resource for enhancing placental barrier models, advancing research and therapeutic assessment in pregnancy, and broadening the breadth of uses for hiPSC-derived trophoblasts [8].

2. CONCLUSION AND PERSPECTIVES

The science of tissue engineering is dynamic and self-renewing, offering potential therapeutic benefits for a range of illnesses. This study investigated the rol of human chorion cells in tissue engineering. Multipotent hCMSCs hold great promise for fetal tissue engineering and regenerative medicine research, as they share nearly the same features as human ESCs. Their use in regenerative medicine is supported by their low immunogenicity, immunomodulatory qualities, and in vitro differentiation capacity. This is especially relevant in allogeneic transplantation settings and for diseases where immunomodulatory properties may have therapeutic effects, such as in cases of inflammation.

The placenta is also a highly desirable tissue, as it is typically discharded after birth making it abundant. Additionally, obtaining it is safe, non-invasive, and presents no ethical issues [30]. Placental studies can provide further insight into reprogramming potantiel of extra-embryonic tissues related to human generation. Compared to other types of stem cells, multipotent hCMSCs offer several unique advantages. First of all, fetal progenitors, which are more versatile and have shorter telomeres than adult cells, can be used to create hCMSCs. Large volumes of growth variables are produced by hCMSCs, which also function as potent paracrine modulators that are resistant to carcinogenesis. A significant numbers of essential hCMSCs can be produced, which may be important for the development of pharmaceuticals.

It is clear from this review that placental chorion cells hold significant potential for tissue engineering. Additionally, this work proposes using placental chorion cells as a substitute source for the induction of pluripotent stem cells from fetal-derived somatic tissues. This approach may expand access to a larger cell supply while reducing the moral and practical challenges related to the use of embryonic stem cells. Using these cells could accelerate the development of patient-specific medications, offering more attractive and cost-effective treatment alternatives.

Further research on this topic of study is necessary. To fully comprehend placental chorion cells' tissue compatibility, separation ability, and long-term impacts, additional studies are needed. Moreover, it remains unclear whether these cells' advantages in reducing inflammation and promoting repair/regeneration result from their isolation or from paracrine effects on surrounding tissue. Incorporating these cells into therapeutic contexts will require more preclinical and clinical considerations. This important information will surely aid in the successful creation of advantageous treatments based on the utilization of placental cell transplantation, as well as showing the safety and effectiveness of the procedure.

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