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Directed Differentation of Human Embryonic Stem Cells in Combination of Biomaterials

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1. Introduction

Human embryonic stem cells (hESC) are derived from the inner cell mass of preimplantation embryos and defined by their extensive self-renewal capacity and their potential to differentiate into any cell types of three germ layers (ectoderm, mesoderm and endoderm). Since the first hESC line was established in the year of 1998, much progress has been made on studies of hESC culture, cell line establishment, and directed differentiation.

2. Culture system of hESC

Culturing hESC in vitro requires not only keeping their multiplication and undifferentiated diploid condition but also maintaining their potential to differentiate into three germ layers. hESC lines are traditionally derived and maintained on mouse embryonic fibroblasts (MEF); even now using MEF as feeder is one widespread way that scientists adopt for hESC cultures. Because of the xenogeneic property of MEF and rapid senescence, people began to search for various kinds of human fibroblasts as feeders and the use of them has been suggested as a plausible alternative. At present, there have developed many kinds of human cell feeders and attempted feeder-free (and serum-free) culture system, non-conditioned medium culture system.

2.1 Feeder-containing culture system

As one of the earliest used feeders for hESC, MEF promotes hESC proliferation by secreting mitogenic factors like fibroblast growth factor (FGF) and inhibit hESC differentiation by secreting differentiation inhibiting factors like leukaemia inhibitory factor (LIF). However, not only MEF enter senescence rapidly, but also need prepare primary MEF cells uninterruptedly. Moreover the use of MEF and other components of animal origin in the culture media for hESC substantially elevates the risk of contaminating these cell lines with infectious agents of animal origin, thereby severely limiting their potential for clinical application[1]. As the therapeutic potential of hESC lies in the transplantation of differentiated cell types for various degenerative diseases, it is important to eliminate potential xenogeneic contamination[2]. Otherwise, hESC in prolonged culture in vitro may cause higher karyotype instability and uncontrollable spontaneous differentiation phenomenon, which also confine manipulation in clinical application[3]. Accordingly, many research groups attempt to use various kinds of human-source cells as feeders, such as

human embryo fibroblast[4], adult fetal muscle fibroblast, adult skin and adult fallopian tube, human marrow stromal cells (hMSC)[5], human foreskin fibroblast (hFF)[6], human placenta-derived feeder (HPC)[7], human amniotic epithelial (HAE)[8], and post natal human fibroblast.

The number of colonies, number of cells produced, and cell survival rates, all of these indexes are significantly higher on MEF than on human feeder cells ($P < 0.01$) and the number of AP-positive colonies and cell quantity are also significantly higher on hMSC than on hFF ($P < 0.01$). It implies that hMSC is superior to hFF in supporting the proliferation of hESC[9]. SNUhES3 (hESC line) cultured on MEF for 50 passages display three chromosomal abnormalities: t(3:5) in the 10th, trisomy 12 in the 20th, and complex of t(3:5) and trisomy 12 in the 30th passage. In the HPC group, only one chromosomal abnormality is noted at the 30th passage: t(3:5)[7].

In another study, people derivatized FGF2 expressing germ layer derived fibroblast cells (GLDF) from hESC lines[2]. These feeder cells with fibroblast cells-like properties maintained the properties of hESC in prolonged culture over 30 passages. Furthermore, these GLDF cells could secrete FGF2 to maintain pluripotency of hESC cultures even in the absence of supplemental FGF2. Otherwise human embryonic lung fibroblasts (hELF)[10] overexpress LIF that result in the higher expressing of hESC multipotency genes Oct4 and Nanog.

Choosing human-derived cells as feeders solves the puzzle of biological safety in some degree, but need to prepare and identify the feeder uninterruptedly. Moreover, the growth cycle is finite, the manipulation is elaborate, all of this limite hESC large amplification and stable passage, bring disadvantages to large-scale culture of hESC[11]. To overcome this constraint, there has developed feeder-free culture system. It may not only offer chance for hESC large-scale culture but also accelerate the step of clinical application.

2.2 Feeder-free culture system

In order to simplify the culture of hESC, several research groups have engaged to establish the feeder-free culture system. Unlike feeder-based cultures, which require the simultaneous growth of feeder and stem cells, resulting in mixed cell populations, stem cells grown on feeder-free systems are easily separated from the surface, presenting a pure population of cells for downstream applications. The conditioned medium is prepared by adding proliferation promoting factors and differentiation inhibiting factors, or cells that secrete the above factors are used. Two main strategies are adopted: (1) using conditioned medium (CM) and culture dishes coated with extracellular matrix as matrigel, laminin or fibronectin; (2) feeder-free and serum-free culture system, in which hESC are cultured in special medium containing serum replacement (SR) and various kinds of growth factors promoting hESC self-renewal.

It has been successful to culture hESC using feeder-free system with matrigel or laminin, but MEF conditioned medium is required which may propagate infectious agents of animal origin to hESC[12]. Besides, matrigel is a mixture derived from murine tumors, containing many extracellular matrix and various kinds of growth factors. For this reason, the ideal feeder-free cultures should deploy xeno-free extracellular matrix and conditioned medium.

A novel feeder layer-free culture system for hESC, has been presented. The defined culture system is based on serum replacement, a combination of growth factors including

transforming growth factor β1 (TGFβ1), LIF, bFGF and fibronectin matrix[13]. BMP-11/GDF-11 and Myostatin/GDF-8 are both members of the TGF-β superfamily that can activate SMAD2/3phosphorylation via the type I receptors ALK4, ALK5, or ALK7, ultimately maintain hESC undifferentiated, maintain POU5f1, NANOG, TRA-1-60, and SSEA4 normally expression[14]. BMP4 synergizing with LIF can maintain mouse ESC self-renewal, while BMPs alone induce hESC differentiation. hESC cultured in unconditioned medium (UM) are subject to high levels of BMP signaling activity. The BMP antagonist noggin synergizes with bFGF to repress BMP signaling and sustain undifferentiated proliferation of hESC in the absence of fibroblasts[15].

Genetic manipulation of hESC is important for both present research and future commercial applications. Under the feeder-free and serum-free conditions, plasmid transfection, virus infection, and siRNA transfection are highly effective. Stable genetically modified hESC lines can be generated with these genetic manipulations without loss of pluripotency or differentiation potential. The majority of lines generated in this system display a normal karyotype[16]. Immunofluorescence microscopy and quantitative PCR analyses of hESC under feeder-free conditions have illustrated with new results on cellular localization of transcriptional factors and components of the Hedgehog, Wnt, and PDGF signaling pathways to primary cilia in stem cell maintenance and differentiation[17].

3. Directed differentiation of hESC

Upon differentiation, hESC can give rise to a variety of cell types, including nerve cells, cardiac muscle cells, endothelial cells, hematopoietic cells, and insulin-producing beta cells, highlighting the importance of these cells in studying the developmental mechanisms and the potential in transplant therapy.

3.1 Neural cells

Studies indicated that neuroectodermal precursors in neural rosettes could be induced by replating EBs into the DMEM/F12 medium containing insulin, transferrin (TFN), corporin, putrescine, liquemine, FGF2. Immunostaining of neuroectodermal cells in rosettes for early neural markers Sox1 and nestin, and the neuronal marker TuJ1, showed that the radially organized columnar neuroepithelial cells coexpressed Sox1 and nestin[18]. All these hESC-derived neural progenitor cells could differentiate into neurons, astrocytes and oligodendrocytes.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive and selective loss of dopaminergic (DA) neurons in the midbrain substantia nigra[19]. The prevailing pharmacological strategy has side effects over time, therefore, much attention has been focused on the transplantation of DA-synthesizing cells. Studies showed that after differentiation of passage 2 cells, 30–50% of the total cells derived from hESC were Tuj1-positive neurons, among the Tuj1-positive neuronal population 64–79% of the cells expressed TH[20].

For the purpose of applying the hESC to PD, many researchers have tried to develop protocols increasing the purity of DA neurons. Myung Soo Cho, etc [21] introduce a method that hESC give rise to functional tyrosine hydroxylase-positive (TH^+) neurons up to nearly 86% of the total hESC-derived neurons. The most unique feature of this method is the generation of homogeneous spherical neural masses (SNMs) from the hESC-derived neural precursors, and it only takes two weeks to induce of DA neurons from SNMs.

hESC-derived DA nerons and neural progenitors raise Ca^{2+} from intra- and extracellular compartments in response to depolarization, glutamate, ATP and dopamine D_2 receptor activation, while cAMP is elevated in response to forskolin and 3-isobutyl-methylxanthine[22]. Analysis of hESC and hESC-derived neural stem cell nuclear extracts revealed an increased expression of Reptin52 in neurosphere nuclei[23] that serves a pivotal regulatory role in nuclear activities such as transcription regulation and histone modification.

The development of stem cell-based neural repair strategies requires detailed knowledge on the interaction of migrating donor cells with the host brain environment. hESC-derived glial precursors (ESGPs) transduced with a retrovirus encoding the polysialyltransferase STX exhibit overexpression of polysialic acid(PSA) which is a carbohydrate polymer attached to the neural cell adhesion molecule (NCAM) [24]. Chemotaxis assays show that overexpression of PSA results in an enhanced chemotactic migration of these cells toward gradients of a variety of chemoattractants, including FGF2, platelet-derived growth factor (PDGF), and brain-derived neurotrophic factor (BDNF), and this effect is mediated via the phosphatidylinositol 3′-kinase (PI3K) pathway.

3.2 Hematopoietic stem cells

The most common human cell-based therapy applied today is hematopoietic stem cell (HSC) transplantation[25]. Human bone marrow, mobilized peripheral blood, and umbilical cord blood are considered as the major sources of transplantable HSCs, but both compatibility between donor and recipient and required quantity limit their clinical application. In contrast, hESC have been shown to differentiate into the hematopoietic cell fate, providing an alternative source of transplantable blood cells. hESC-derived hematopoietic stem cells which emerge from a subset of embryonic endothelium expressing PECAM-1, Flk-1, and VE-Cadherin, but lacking CD45 (CD45negPFV) show similar clonogenic capacity and primitive phenotype to somatic sources of hematopoietic progenitors and possess limited in vivo repopulating capacity in immunodeficient mice. A subset of hESC-derived $CD45^+$ hematopoietic cells coexpress CD34 and show progenitor function in colony-forming units assays. However, compared to HSC from fetal blood (FB) or cord blood, these hESC-HSC display distinct functional properties, including poor repopulation ability, impaired differentiation and lack of homing[26]. Only 2.4% of differentially expressed transcripts were common for FB–HSCs and candidate hESC-HSCs[26], suggesting a completely different molecular signature for HSCs isolated from two different in utero ontogeny stages. Several key hematopoietic transcription factors, such as RUNX3, TAL1, VAV1, LMO2, AML-1, and c-mac, apoptosis and cycle regulators, such as CDC42, CDC27, CyclinD3 and CDK4, and cell aggregation and homing genes may contribute to explain the functional differences between hESC-HSCs and FB-HSCs. Importantly, the gene expression profiling study revealed that developmental signaling pathways such as Notch, Wnt, BMP, Shh are involved in HSC self-renewal and hematopoietic specification.

Several complementary methods are used to demonstrate canonical Wnt signaling that is important for development of hESC-derived cells with both hematopoietic and endothelial potential[27]. The development of cells with hematoendothelial potential decreases dramatically when treated with dickkopf1 which inhibits Wnt signaling. In addition, activation of the canonical Wnt signaling pathway in hESC by coculture with stromal cells that express Wnt1, but not use of noncanonical Wnt5-expressing stromal cells, results in an

accelerated differentiation and higher percentage of $CD34^{bright}CD31^{+}Flk1^{+}$cells at earlier stages of differentiation.

3.3 Mesenchymal stromal cells

Mesenchymal stromal cells (MSCs), originally isolated from adult bone marrow (BM), have also been derived from hESC, either through coculturing with the OP9 murine BM stromal cell line or directly from ESCs cultured without feeder cells[28,29]. The simultaneous generation of $CD73^{+}$ MSCs along with $CD34^{+}$ hematopoietic cells from HESC has been shown when they are cocultured with OP9 murine stromal cells[28]. hESC–derived MSCs show spindle-shaped fibroblast-looking morphology, exhibit similar cell surface marker CD29, CD44, CD54, CD73, CD90 and CD105, but negative for CD34, CD45, and the endothelial marker CD31, characteristics when compared to BM-derived MSCs, and could differentiate into osteocytes, adipocytes, and chondrocytes.

3.4 Insulin-producing β cells

Type I diabetes is a disease in which β-cells of the pancreas are destroyed by an autoimmune mechanism[30]. Use of exogenous insulin to treat diabetes is life-saving; however, it does not truly mimic the body's natural response to blood glucose[31]. Islet cell transplantation is currently considered to be an important method of therapy[32]. But the scarcity of islets prevents this therapy. The isolation of hESC introduced a new prospect for obtaining a sufficient number of β cells for transplantation. Segev et al. present a method for forming immature islet-like clusters of insulin-producing cells derived from hESC[33]. Seven-day-old EBs were first cultured and plated in insulin-transferrin-selenium-fibronectin medium (ITSF) about a week, followed by medium supplemented with N2, B27, and bFGF. At the next stage, bFGF was removed and nicotinamide was added, and the total glucose concentration in the medium was reduced. After 4 days of culture, the formation of clusters exhibited higher insulin secretion and had longer durability than cells grown as monolayers. Gen-hong Mao[34] demonstrates a five-stage protocol with adding exendin-4 instead of nicotinamide and generates islet-like cells from human embryonic stem (ES) cells. Immunofluorescence analysis revealed that most c-peptide positive cells coexpressed inlusin, PDX-1 (pancreas duodenum homeobox-1), glucagon, somatostatin or pancreatic polypeptide. Insulin and other pancreatic β-cell-specific genes were all present in the differentiated cells.

Following the illumination of hESC differentiation mechanism and successfully culturing various kinds of terminal differentiated cells, tissues and organs, it may apply functioning cells of hESC-source in replacement therapies.

4. hESC coupled with biomaterials

Tissue engineering is the study of the growth of new connective tissues or organs, from cells and a collagenous scaffold to produce a fully functional organ for implantation back into the donor host. Scaffolding can be used as a cell support device upon which cells are seeded in vitro; cells are then encouraged to lay down matrix to produce the foundations of a tissue for transplantation. Tissue engineering scaffolds are designed to influence the physical, chemical and biological environment surrounding a cell population[35]. Many matrix materials that are in application of current research are mainly natural materials, synthetic polymer and biodegradable materials, ceramic materials and their complex formed between the composite materials. Natural biological materials, compared with synthetic materials,

have many advantages, the abundant source, low cost, and with good biocompatibility, and that some materials have pore structure of natural systems. Currently, the mainly applied materials are collagen (type I collagen in the main), gelatin, chitin, chitosan, natural coral and its derivatives. Now the synthetic biodegradable polymer materials that extensively used are mainly polylactic acid (PLA), polyglycolic acid (PGA) and various copolymers between them. These materials are bulk-degrade by hydrolysis, providing a controllable drug release and degradation profile to match tissue in-growth. The main structures of PLA, PGA and their copolymers used in tissue engineering applications are in the form of fibrous, porous foam and tubular structure.

4.1 Electrospun fibrous scaffolds

Electrospun fibrous scaffolds can be prepared with high degree of control over their structure creating highly porous meshes of ultrafine fibers that mimic the natural three-dimensional environment of the in vivo extracellular matrix (ECM), and are amenable to various functional modifications targeted towards enhancing stem cell survival and proliferation, directing specific stem cell fates, or promoting tissue organization[36]. Evidence shows that of electrospun biodegradable polymers as scaffolds not only enhances the differentiation of mouse ES cells into neural lineages but also promotes and guides the neurite outgrowth[37]. Cultivation of hESC on electrospun fibrous polyurethane scaffolds also proved successful and neuronal differentiation was observed via standard immunocytochemistry[38]. Scanning electron micrographs confirmed neurite outgrowth and connection to adjacent cells, as well as cell attachment to individual fibers of the fibrous scaffold. Scaffold architecture has been shown to modify the response of cells and subsequent tissue formation, as demonstrated by the generation of mineralization fronts in specific regions of scaffolds. Nano to microscale topography has been demonstrated to affect cell behavior by modification of cytoskeleton arrangements.

Although complex structures are formed, the mechanism of adhesion of hESC to the surfaces of the scaffolds remains largely unknown. Steven Y. Gao[39] achieved the efficient adhesion of pluripotent hESC to 3D PLGA scaffolds. In the 2-D PLGA model, the scaffold surfaces were coated with laminin and flow cytometry analysis revealed that almost all of the pluripotent single cells expressed the integrin α6, with a small percentage also expressing α3β1, which facilitates adhesion to laminin. In the 3-D enviroment, the results were similar to those gained from the 2-D model, which suggests that the adhesion of pluripotent hESCs is mainly affected by the surface properties that they adhere onto rather than the geometric properties of the culture system.

4.2 Porous foam scaffolds

Porous biodegradable polymer scaffolds can be used to support ES cells because they represent a promising system for allowing formation of complex 3D tissues during differentiation[40]. The scaffolds which consist of a 50/50 blend of PLGA and PLLA could support hESC that remain viable for at least 2 weeks in vivo. Two weeks after transplantation of hESC-scaffold constructs into SCID mice, defined epithelial tubular structures and neural tube-like rosettes were observed. In addition, when cultured in vitro with both NGF and NT-3, enhanced numbers of neural structures and staining of nestin and βIII-tubulin were observed with hESC-seeded polymer scaffolds[41]. NGF and RA preferentially favor the differentiation toward ectodermal and mesodermal lineages[42]. Subcutaneous transplantation of PLGA scaffolds seeded with the hESC-derived islet-like

cells or cell transplantation under kidney capsules for further differentiation in vivo could improve 6 h fasted blood glucose levels and diabetic phenotypes in streptozotocin-induced diabetic SCID mice[34]. More interestingly, blood vessels of host origin can be observed, invading the cell–scaffold complexes. This suggests scaffolds can serve as vehicles for islet-like cell transplantation.

Other study has certificated that 3D porous alginate scaffolds provide a conductive environment for generation of well-vascularized EBs from hESC[43]. EBs can be generated directly from hESC suspensions within 3D porous alginate scaffolds. The alginate scaffold pores which provide a confining environment enable efficient formation of round, small-sized EBs with a relatively high degree of cell proliferation and differentiation, and induce vasculogenesis in the forming EBs to a greater extent. Recent study demonstrated that 3D porous natural polymer scaffold, comprised of chitosan and alginate, could support sustained self-renewal of hESCs without the support of feeder cells or conditioned medium[44].

4.3 Other scaffolds

Scaffolds can be used as a growth factor/drug delivery device[35]. This strategy involves the scaffold being combined with growth factors, so upon implantation cells from the body are recruited to the scaffold site and form tissue upon and throughout the matrices. Recently, vascular progenitor cells as well as endothelial and smooth muscle cells have been isolated from hESC encapsulated in bioactive hydrogel-based scaffolds combined with immobilized regulatory factors: a tethered RGD peptide and microencapsulated VEGF165[45]. Others have investigated the functions of hESC-derived neural progenitor cells (hESC-NPC) after transplanted in collagen scaffolds supplemented with growth factors[46] and reveal that implantation of hESC-NPC into the spinal cord with collagen scaffold improved the recovery of hindlimb locomotor function and sensory responses in an adult rat model of SCI.

Tzu-I Chao, et al.[47] have reported that 2D thin film scaffolds composed of biocompatible polymer grafted carbon nanotubes (CNTs), can selectively differentiate hESC into neurons while maintaining excellent cell viability. According to fluorescence image analysis, neuron differentiation efficiency of polyacrylic acid grafted CNT thin films is greater than that on polyacrylic acid thin films. Other comparison has been done between silk and chitosan scaffolds in chondrogenesis of human cell sources[48]. After 4 weeks of cultivation, hESC-derived MSCs were promising for chondrogenesis, particularly in the silk scaffolds with BMP6. The polyurethane acrylate (PUA), fabricated into nanoscale ridge/groove pattern arrays with its surface treated in oxygen plasma for 60s Results showed that after alone[49] can effectively and rapidly induce the differentiation of hESC into a neuronal lineage without the addition of any biochemical or biological agents.

Future applications of those versatile scaffolds platform to human embryonic and induced pluripotent stem cells for functional tissue repair and regeneration will further expand its potential for regenerative therapies.

5. Prospect of HESC

Expecting to use hESC largely and safely in clinical, there are a lot of works to do. hESC differentiation is typically stimulated via generation of EBs and lineage commitment of

individual cells depends upon numerous cues throughout the EB environment, including EB shape and size. Common EB formation protocols, however, produce a very heterogeneous size distribution, perhaps reducing efficiency of directed differentiation[50]. Some scholars have developed a 3-D microwell-based method to maintain undifferentiated hESC cultures for weeks without passaging using physical and extracellular matrix patterning constraints to limit colony growth. Microwell culture also permits formation of hESC colonies with a defined size, which can then be used to form monodisperse EBs. When cultured in this system, hESC retained pluripotency and self-renewal, and were able to be passaged to standard unconstrained culture conditions.

Successful clinical implementation of tissue engineering products generated from ESCs would require a controlled reproducible culture system for the expansion of the cells and differentiation into functional tissues. It is a marvelous scenario which involve combination of the bioreactor expansion and subsequent differentiation of the ESCs to generate the specialized cell types. N. I. zur Nieden[51] showed that suspension bioreactors which can be adapted for long term culture could be used in the regulated large-scale expansion of highly pluripotent murine ESCs. The ESC cultures remain highly undifferentiated, when serially passaged in suspension bioreactors for extended periods. Otherwise different growth factors have different effects on cell proliferation and differentiation[52]. BMPs have previously been shown to induce hESC differentiation, in apparent contrast to mouse embryonic stem (ES) cells, in which BMP4 synergizes with LIF to maintain self-renewal. The BMP antagonist noggin synergizes with bFGF to repress BMP signaling and sustain undifferentiated proliferation of hESC in the absence of fibroblasts or CM. These findings suggest a basic difference in the self-renewal mechanism between mouse and human ES cells.

Stem cells and human tissue engineering are two hot spots. Many scholars attempt to combine hESC with tissue engineering. Although transplantation of ES cells-derived neural progenitor cells has been demonstrated with some success for either spinal cord injury repair in small animal model, control of ES cell differentiation into complex, viable, higher ordered tissues is still challenging[37].

Silk fibroin (SF), the core structural protein of *Bombyx mori* silkworm silks, has widely been studied as biomaterials in tissue engineering and regenerative medicine due to the biocompatibility, impressive mechanical property, high yields through sericulture and controllability of degradability, as well as the versatility in processing, the availability of different morphologies and the ease of sterilization and surface modification of these SF-based materials. While a number of studies detailed the ability of SF to support the attachment and growth of a variety of human cell types and the promising applications of SF scaffolds in wound healing and in bone, cartilage, blood vessel, tendon, or ligament tissue engineering, there is little information about the interaction of astroglial and neuronal cells with SF-based scaffolds.

Among all the silk materials in different morphologies, of great interest are the electrospun nonwoven SF nanofibers or nets that mimic the nanostructured components of the extracellular matrix and are supposed to provide more surface area and rougher topography for cell attachment. Upon implantation these nanofibrous SF scaffolds might ameliorate the conditions at the injury sites via the formation of the oriented network of astroglial and neuronal cells by arranging these cells along the nanofibers and guiding axonal outgrowth, and finally prevent the formation of physical and molecular barriers to the CNS repair. Nonwoven SF nanofibers were studied with a variety of cells, but little information is

hitherto available with neurons, astrocytes and their progenitors, the main cell types participating in the glial scaring.

Tussah silk is a type of wild silks, and the chemical structure, molecular conformation and physical properties of tussah silk fibroin (TSF) have been extensively studied. In contrast to *Bombyx mori* silkworm silk fibroin (SF), the amino acid composition of TSF is characterized by more Ala, Asp and Arg contents, and less Gly, and the presence of tripeptide sequence Arg-Gly-Asp (RGD), which is known to promote cell attachment and thus might make TSF an even more promising biomaterial for use in biomedical applications and tissue engineering.

In an attempt to develop strategies for fabricating nerve implants, using the regenerated SF, TSF, or blends of both at different proportions, that could be applied in vivo as the cell substrates to control the arrangement of astroglial cells and guide the axon growth, we prepared nonwoven nanofiber scaffolds by electrospinning and analyzed the behavior of astrocytes, neurons and neural progenitor cells, isolated from newborn rat cerebral cortex or derived from hESC, on these nonwoven fibroin nanofibers. In particular, we focused on the migration and spreading, in addition to the attachment, viability and proliferation of these cells. Our long-term goal is to identify the optimum conditions in the context of the properties of the nanofiber substrates that may allow for the manipulation of astroglial cells and ameliorate the nonpermissiveness of the CNS injury sites upon implantation.

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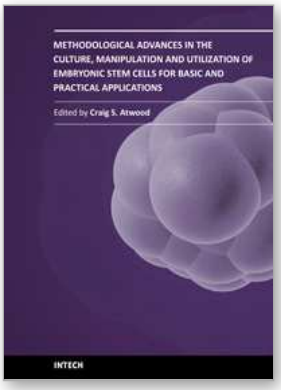

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Pluripotent stem cells have the potential to revolutionise medicine, providing treatment options for a wide range of diseases and conditions that currently lack therapies or cures. This book describes methodological advances in the culture and manipulation of embryonic stem cells that will serve to bring this promise to practice.

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