Journal of Bionanoscience
Vol. 9, 13–21, 2015

REVIEW

Impact of Nanotechnology in Induced Pluripotent Stem Cells-driven Tissue Engineering and Regenerative Medicine

Murugan Ramalingam[1, 2, *] and Deepti Rana[1]

[1] *Centre for Stem Cell Research (CSCR), A unit of Institute for Stem Cell Biology and Regenerative Medicine-Bengaluru, Christian Medical College Campus, Vellore 632002, India*
[2] *WPI-Advanced Institute for Materials Research, Tohoku University, Sendai 980-8577, Japan*

The aim of this article is to investigate the current trends and impact of nanotechnology in induced pluripotent stem cells (iPSCs)-driven tissue engineering and regenerative medicine. The iPSCs are considered as one of the potential cell sources for tissue engineering applications due to their self-renewal and differentiation abilities. However, the key to realize their full potential in tissue engineering requires a deep understanding of the iPSCs biology and their cellular interaction with three-dimensional (3D) scaffolds, which support and regulate the cellular growth and function, in conjunction with signaling molecules. At the cellular and molecular level, nano-scale features play an important role in controlling cell behavior and other physiological functions of iPSCs. Therefore, nanomaterial-based scaffolds have tremendous impact in iPSCs-driven tissue engineering and regenerative medicine. Nanomaterials have been proved to serve as a scaffolding system for tissue engineering, as a carrier system for delivery of cells and genes, and as a marker system for imaging and tracking of iPSCs. In this article, we therefore discuss briefly the impact of nanotechnology on cell behavior and iPSCs-driven tissue engineering and regenerative medicine applications with their recent challenges and advancements.

Keywords: Nanotechnology, Induced Pluripotent Stem Cells, Biomaterial, 3D Microenvironment, Tissue Regeneration.

CONTENTS

1. INTRODUCTION

Tissue engineering aims to develop functionalized tissues and organs for repair and restoration of defective tissues and organs of human body with the help of cells and engineered constructs called scaffold.[1] Scaffold plays a key role in tissue engineering by providing a structural support for the cells to accommodate and guide their growth in the 3D space into a specific tissue or organ. Stem cells are a kind of cells that has a remarkable potential to self-renew and differentiate into many different tissue types; thereby they are considered as an important cell source for tissue engineering. Combining stem cells with biomaterial scaffolds provides a promising strategy for engineering tissues and organs, as well as delivery of cells. The development of a viable construct involves a suitable supply of cells that shows non-immunogenic and proliferative behavior; are easy to harvest, and possess ability to differentiate into a variety of cell types with specialized functions. For patients with extensive end-stage organ failure, and with limited proliferative capacity in culture, stem cells are envisioned as being an alternative source of cells.

Stem cells encompass a large class of cell types, which includes fate-restricted multipotent adult stem cells, embryonic stem cells (ESCs) and recently discovered induced pluripotent stem cells (iPSCs). These cells can be autologous, allogeneic or xenogeneic in nature. Among them, iPSCs hold significant promise for generating engineered tissues and organs owing to its ESCs-like state and thus it has attracted the stem cell community. The human iPSCs have biological similarities with human ESCs with

*Author to whom correspondence should be addressed.

respect to their morphology, differentiation potential and molecular signature[2] surpassing ESCs limitations, which makes it a potential replacement of ESCs in therapeutic applications. The iPSCs have the potential for generating patient-specific cells with high pluripotency by reprogramming the patient's own-cells and thereby can reduce the chances for immunological rejection. Additionally, there are no or less ethical concerns associated with the iPSCs in comparison to ESCs. It also excludes the need for invasive procedures to obtain pluripotent cells because of the vast availability of reprogrammable cell types.

Scaffolds, on the other hand, are also an important factor for determining the success of the tissue engineering. Due to the several merits associated with iPSCs-seeded tissue engineered scaffolds, in terms of their structural and functional properties that can mimic the native extracellular matrix (ECM), these kinds of iPSCs-seeded engineered scaffolds have applications in different areas of tissue engineering such as bone, cartilage and neural. For instance, Hoveizi et al., have recently compared the cell adhesion and proliferation behavior of human iPSCs on polycaprolactone (PCL) electrospun nanofibrous scaffolds with solution-cast film scaffolds, to understand the interactions between ECM mimicking nanomaterials and cells.[3] The results of this study demonstrated that the nanofibrous scaffolds showed better support for the attachment and proliferation emphasizing on the sensing ability of human iPSCs in regard to the physical properties and chemical composition of the nanomaterial.[3] Similarly, Liu et al., have employed the potential of iPSCs in 3D PCL/gelatin scaffolds for chondrogenesis and articular cartilage defect restoration.[4] This study demonstrated higher expression levels of chondrogenic markers in iPSCs than the control groups. All these experimental examples, and others, clearly indicate the impact of scaffolding system in controlling cellular behavior of iPSCs.

In the recent years, nanotechnology has added a whole new dimension in engineering materials in the form of

Murugan Ramalingam is Professor at the Centre for Stem Cell Research (a unit of the Institute for Stem Cell Biology and Regenerative Medicine-Bengaluru), Christian Medical College Campus, India. Concurrently he is Adjunct Professor at the Tohoku University, Japan. Prior to joining the CSCR, he was Associate Professor of Biomaterials and Tissue Engineering at the Institut National de la Santé et de la Recherche Médicale, Faculté de Chirurgie Dentaire, Université de Strasbourg, France. He has worked at the WPI Advanced Institute for Materials Research, Japan, as an Assistant Professor. He has also worked at the National Institute of Standards and Technology (NIST) and the National Institutes of Health (NIH), under the U.S. National Academies Associateship program. He received his Ph.D. in Biomaterials from the University of Madras. He has also undergone training in Ethical and Policy issues on Stem Cells from Harvard University, USA, and in Operations Management from the University of Illinois-Chicago. His current research interests are focused on the development of multiphase biomedical materials, through conventional to nanotechnology to biomimetic approaches, microfabrication, cell patterning, stem cell differentiation, tissue engineering and drug delivery. He is the author of over 200 publications, including peer-reviewed journal papers, conference proceedings, book chapters, authored books, edited books, and patents relevant to biomaterials, stem cells, and tissue engineering. His current h-index is 23 with over 4300 citations. He has organized several international conferences and chaired Biomaterials, Nanobiotechnology, Stem Cells and Tissue Engineering sessions. He also serves as a board member of several international scientific and research committees in various public and private bodies and grant reviewer of various international funding agencies. He serves on the editorial boards of multiple biomaterials and tissue engineering-related journals, including as the Editor-in-Chief of the Journal of Biomaterials and Tissue Engineering, the Journal of Bionanoscience and the American Journal of Stem Cell Research. He is a recipient of several prestigious fellowships and awards, including CSIR Fellowship (India), SMF Fellowship (Singapore), NRC National Academies Fellowship (USA), Nationale Professeur des Universités (France), Fellow of Institute of Nanotechnology (UK) and Fellow of Royal Society of Chemistry (UK).

Deepti Rana is a Junior Research Fellow at the Centre for Stem Cell Research, Christian Medical College, Vellore, India. Her research interests include the development of multi-scale (nano to micro to macro) biomaterials for translational stem cell research.

scaffolds suitable for iPSCs-driven tissue engineering. The scaffolds made of nanomaterials play a critical role in accommodating cells and guide them to differentiate into a specific tissue during regenerative process. The cellular behaviors, such as adhesion, proliferation, migration and differentiation, of cultured cells can be controlled by manipulating the structure and properties of microenvironment where the seeded cells are intended to grow. Nanotechnology can be used to impart the structure and properties of native microenvironment within the scaffolding system in order to enhance the cellular growth and function. The application of nanotechnology is not only limited to tissue engineering but it can also be extended to other fields such as labeling, imaging and tracking of iPSCs, genetic modification of iPSCs and manipulating the microenvironment/niche of iPSCs.[5–7]

Considering the aforementioned impact of nanotechnology in the field of iPSCs, in this article, the authors have focused their attention to concisely review the different types of nanomaterials, their interactions with iPSCs and to evaluate their use in combination with iPSCs suitable for various biomedical applications, which includes iPSCs-driven tissue engineering, iPSCs labeling and tracking, iPSCs generation methods, and manipulation of iPSCs microenvironment. The authors do not suggest that this is the only choice of cells available for tissue engineering and regenerative medicine, but the key intention is to stimulate research on iPSCs in the context of nanomaterial-driven tissue engineering and to evaluate their full potential, in terms of cellular growth and function, as an alternate cell source for tissue regenerative medicine. The article is expected to be useful for readers to gain insights into current trends and impact of nanotechnology in iPSCs research.

2. BASICS OF INDUCED PLURIPOTENT STEM CELLS

The iPSCs are adult cells that have been genetically reprogrammed to an ESCs-like state by being forced to express genes and factors important for maintaining the defined properties of ESCs.[8] The iPSCs are considered as one of the potential cell sources for tissue engineering due to their self-renewal and differentiation abilities. At the outset, Yamanaka and team proposed that the iPSCs have properties similar to ESCs, which can be derived from mouse or human fibroblasts using four transcription factors including Oct3/4, Sox2, c-Myc, and Klf4, under ESCs culture conditions.[9] Later, Thomson and team reported the four factors (OCT4, SOX2, NANOG, and LIN28) are sufficient to reprogram human somatic cells into pluripotent stem cells, which has the characteristics of ESCs.[10] However, ethical issues have been raised in usage of human embryos and problems related to the tissue rejection after being transplanted to patients. To avoid these issues, pluripotent cells are directly derived from the patient's own cells and these novel stem cells were specified in the name of iPSCs. In addition, the differentiation of iPSCs into functional cells is beneficial for cell-based therapy and also plays an important role in the establishment of patient-specific disease models for drug discovery and development.[11] Besides the overwhelming achievements of reprogramming approach, there are still some unresolved issues that need to be solved out. Some of the limitations of reprogramming approach includes poor transformation rate, risk of developing mutations in the genome as well as cancer induction and sometimes, incomplete reprogramming that can cause danger to the organism.[12] In recent years, nanotechnology has emerged as a new, exciting field into the iPSCs regimes to further enhance the potential of iPSCs-based therapies. Owing to their unique properties, nanomaterials provide new opportunities to solve some of the current limitations associated with iPSCs. For instance, nanotechnology can be applied in the remodeling process of somatic cells or efficient generation of iPSCs and labeling of iPSCs for long-term *in vivo* imaging and tracking applications.[13] Not only nanotechnology has shown great impact on improvements in imaging and tracking techniques, but also there have been several ground-breaking discoveries in iPSCs-driven tissue engineering and regenerative medicine.

3. NANOMATERIALS FOR iPSCs-DRIVEN TISSUE ENGINEERING

Nanomaterials hold great promise as a scaffolding system for the culture of iPSCs and their application in tissue engineering due to their unique functional properties which can be tuned to suit the mechanical and physiological demands of the host tissue by controlling the volume fraction, morphology and arrangement of the reinforcing phase. The general concept of iPSCs-driven tissue engineering involves the culture of isolated/reprogrammed cells from the patient or donor into a scaffolding system, made-up of nanomaterials, that can support the growth and function of the isolated/reprogrammed cells into a specific tissue, which could be transplanted back to the defective site of the patient where tissue regeneration is required (Fig. 1). In this case, cells, scaffolds and bioactive molecules are the key components that determine the success of tissue engineering. The selection of these components is of great importance for the better results of cell-material interactions and cell–cell communications that guides the tissue regeneration and tissue remodeling *in vivo*.

Scaffold made of nanomaterials plays a key role in tissue engineering by providing a structural support and 3D microenvironment to the cells in order to support cell attachment and subsequent tissue development. From biological perspective, cells in the human body resides in a complex mixture of pores, ridges and various components of micro- and nano-featured ECM environment, which

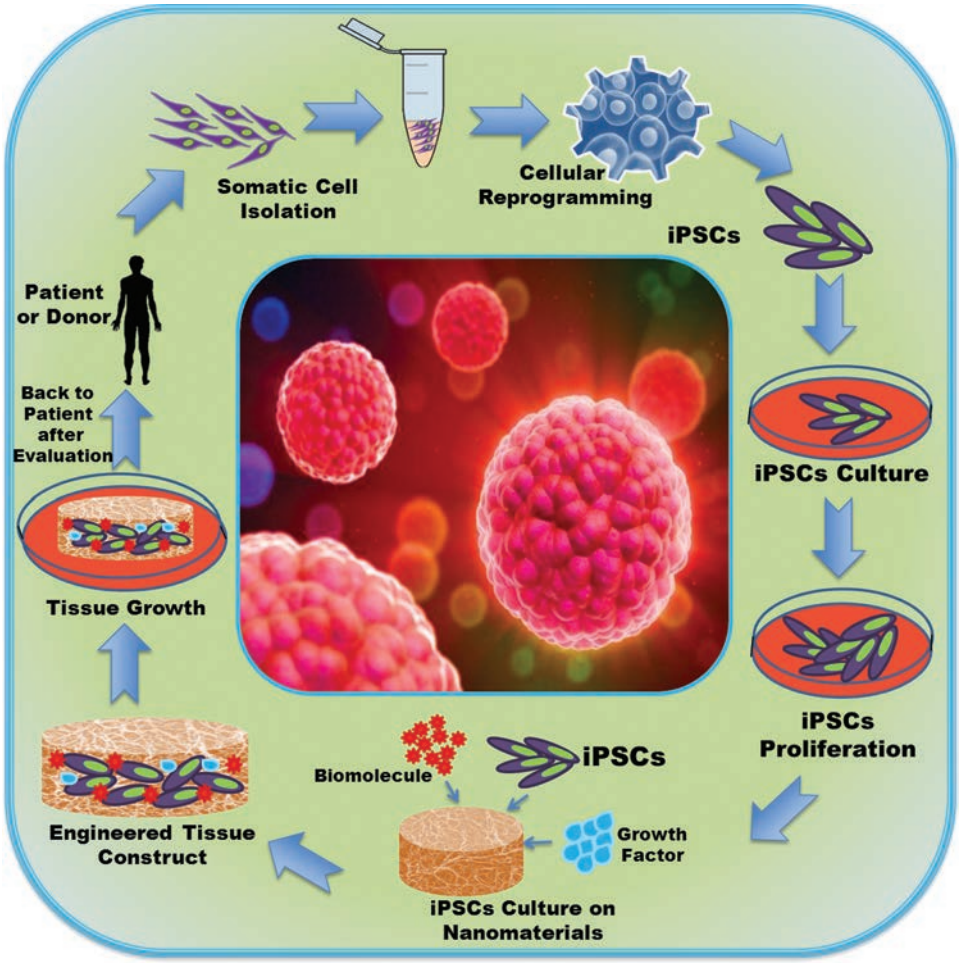

Fig. 1. Schematic representation of concept of tissue engineering approach using iPSCs as a cell source and nanomaterials as an engineered scaffold.

plays a vital role in facilitating cell-matrix interactions and cell–cell communications upon implantation of the engineered graft. Therefore, development of scaffolds with structure and properties matching to the native ECM is essential in order to mimic the microenvironment of native tissue. However, the multiple functions, the complex composition and the dynamic nature of ECM in native tissues make it difficult to mimic exactly. Nevertheless, many types of nanomaterials have been tested worldwide as tissue scaffolding systems.[14–16] Different types of nanomaterials used in various iPSCs-based biomedical applications are listed in Table I. It is evident from the literature survey that most of the nanomaterials are being used as a scaffolding system for tissue engineering, as a carrier system for delivery of cells and genes, and as a marker for imaging and tracking of iPSCs.

Over the years, nanomaterials have moved from merely interacting with the body to influencing biological processes toward the goal of tissue regeneration. Recently, Mohtaram et al. (2014) have investigated the effect of micro- and nano-scale topography on promoting neuronal differentiation of human iPSCs and directing the resulting neuronal outgrowth.[27] Loop mesh and biaxial aligned microscale retinoic acid functionalized-PCL nanofibers were seeded with neural progenitors derived from human iPSCs. From the cell culture study, it was noticed that maximum neurite outgrowth length of these cells occurred on the biaxial aligned scaffolds than the loop mesh topography, giving insight into how physical and chemical cues can be used to engineer neural tissue.[27] This study confirms the importance of the nanoscale features in the designing of scaffolds for directing stem cell differentiation. Evidently, it has been proved that incorporation of various growth factors or bioactive molecules in nano-carrier systems as biological signals can promote the desired differentiation lineage within the seeded stem cells. Since the first contact of the cultured cells is surface of the materials where they had grown, development of an optimal surface engineered nanomaterial is of great importance for the better understanding of cell-nanomaterial interactions and cell–cell communications. In support of the importance of stem cell-nanomaterial interaction, recently it has been reported that precise control of scaffold's structure can provide a more efficient carrier of the seeded stem cells for the regeneration of the damaged tissue. Due to the better cell-material interactions, numerous studies have

Table I. List of various nanomaterials employed for the development of iPSCs-based biomedical applications.

S. No.	Type of nanomaterial	Cells studied	Application	Reference
1.	Poly(beta-amino ester) nanoparticles	Human fibroblast	iPSCs generation	[17]
2.	Vitronectin-decorated polyvinyl alcohol/hyaluronan polysaccharide nanofibers	Human iPSCs	Xeno-free Culture of Human iPSCs	[18]
3.	Plasma-treated polymeric nanofibrous polyethersulfone scaffolds	Human iPSCs	Bone tissue engineering	[19]
4.	350 nm width nanograted Polydimethylsiloxane (PDMS) substrates	Human iPSCs	Neural tissue engineering	[20]
5.	Polyethersulfone nanofibrous scaffold	Human iPSCs	Bone tissue engineering	[21]
6.	PDMS-based microchannel-nanochannel-microchannel (MNM) array	13kbp OSKM plasmid of iPSCs	iPSCs generation	[22]
7.	Poly(D,L-lactide-co-glycolide)/branched polyethyleneimine-DNA nanoparticle	Mouse embryonic fibroblasts	Somatic cell reprogramming	[23]
8.	Cationic bolaamphiphile	Human fibroblasts	iPSCs generation	[24]
9.	Mesoporous silica nanoparticles (MSN) and FITC-conjugated MSN (FMSNs)	Mouse fibroblast-derived iPSCs	Definitive hepatic induction and labeling	[25]
10.	Polyamidoamine dendrimer surfaces with G1, G3 and G5 dendron structure	Human iPSCs	Switching between self-renewal and lineage-commitment	[26]

supported the use of iPSCs-seeded engineered scaffolds in different areas of tissue engineering such as bone, cartilage and neural. All these aspects are described briefly in the following sections.

Bone is a natural nanocomposite made up of organic (collagen) and inorganic (hydroxyapatite (HA)) components arranged in a hierarchical structure ranging from nano- to macro scale. As nanostructures can provide a closer approximation to the native bone architecture, thereby nanomaterials offer a platform to recapitulate the organization of natural ECM for the development of functional bone tissue constructs. Patient-specific bone substitutes can be produced using nanoscale biomaterials and iPSCs technology for variety of bone reconstructive treatments. Bioceramics such as HA and other calcium phosphates (CaP) are some of the important biomaterials in bone tissue engineering. The CaP-based composite materials are widely used for bone grafting due to their bioactive potential (e.g., osteoconductivity and osteointegration). Recently, D'Angelo et al. (2012) produced nanocomposite fibrous mats of poly L lactic acid (PLLA) loaded with different amounts (1 or 8 wt%) of calcium-deficient HA (d-HA) to induce osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBM-MSCs) and murine pluripotent (iPSCs and ESCs) stem cells in the absence of exogenous soluble differentiating agents.[28] Interestingly, it was found that the dispersion of different amounts of d-HA to PLLA resulted into a set of composite materials (PLLA/d-HA) with similar architectures and tunable mechanical properties. The results of the 3 weeks study have confirmed the expression of osteogenic markers on PLLA/d-HA nanocomposite with significant deposition of bone matrix proteins. Murine pluripotent and human multipotent stem cells cultured on neat PLLA scaffold under the same culturing condition were reported to lack osteogenic differentiation. Additionally, electrospun PLLA/d-HAp nanocomposite were found to be independent of the stem cell type. Notably, d-HA concentration of 1 wt% was reported to be sufficient to activate the molecular signals for the onset of differentiation mechanisms. It is likely that for an effective biological responsiveness, the direct interaction is required between the seeded stem cells and PLLA/d-HA substrates. Therefore, this study highlights on the fact that direct interaction of stem cell-polymeric nanocomposite and the mechanical properties acquired by the PLLA/d-HAp nanocomposite play as a key role in osteogenic differentiation.[28] Indeed, it has been proposed that stem cells are able to convert mechanical cues into biochemical signals and in turn can modulate their fate and function, through the process of mechano-transduction.[28] Besides the synthetic origin-based nanomaterials, many other natural origin-based nanomaterials have also been employed to reproduce the biochemical and biophysical simulation of native niche. Interestingly, Wang et al. (2014) have investigated a unique matrix assembled from engineered M13 phage bionanofibers with specific nanotopographical- and versatile signal peptides-based cues to simulate native niche for directing the fate and function of iPSCs.[29] The M13 phage is a virus that specifically infects bacteria and is harmless to human beings, is indeed a bionanofiber (~880 nm long and ~6.6 nm wide). These phage bionanofibers displayed with different signal peptides were assembled into a matrix using a layer-by-layer self-assembly method to present an ordered surface nanotopography. In this method, electrostatic interactions come into play between the cationic poly-L-lysine and anionic phage bionanofibers to achieve the self-assembly of phage matrix. The authors have studied the biochemical (peptide sequence) and biophysical (nanotopography) properties of this unique matrix,

and have found that the resident iPSCs on the phage matrix are first differentiated into mesenchymal progenitor cells and then into the osteoblasts due to the elongation induced by phage nanofibers. It was supported that the cooperative combination of the ordered ridge/groove nanotopography (biophysical cues) and growth factor signal peptide (biochemical cues) can significantly promote the osteoblastic differentiation of iPSCs.[29] These experimental examples, and other studies, demonstrated the efficacy of nanomaterial-based scaffolds in osteogenic differentiation of iPSCs. Though the nanomaterials serve as an effective scaffolding system for bone tissue engineering, further research is required to develop more sophisticated biomimetic nanomaterial scaffolding systems with added levels of complexity to incorporate multi-functionality and to impart osteoconductive-cum-osteoinductive features.

Cartilage tissue engineering is a promising method for the repair and regeneration of cartilage tissues which are damaged or injured by a trauma, osteoarthritis or rheumatoid arthritis.[30] Unfortunately, articular tissues lack self-renewal ability due to its low cell to matrix ratio which in turn limits the natural regenerative potential of the tissue.[31] There are numerous surgical methods that have been developed for the restoration of the cartilage such as drug therapy, micro-fracture, drilling, abrasion arthroplasty, autologous chondrocyte implantation and osteochondral autograft/allograft transplantation. Despite of their wide acceptance, these conventional approaches are also associated with several limitations such as cost effectiveness, inability to regenerate cartilage with mechanical properties matching to the native tissue or sometimes provide only symptomatic relief to the patient. To overcome these limitations, tissue engineering has developed artificial substitutes quite similar to the propoerties of native ECM by using iPSCs and nanotechnology.[32] For instance, Liu et al. (2014) studied the chondrogenic potential of iPSCs cultured in PCL/gelatin nanofibers in order to restore cartilage defects.[33] It was also shown that PCL can provide mechanical strength to the scaffold as even after two months of degradation analysis, no PCL fibres hydrolysis was observed. Hence even though there was 40% decrease in the tensile strength of the degraded fibres yet the young's modulus remained unchanged. The results of the *in vivo* experiment showed higher levels of collagen II, aggrecan and SOX9 expression specific to the cartilage along with sub-chondral bone regeneration. Therefore, due to the nanofiber morphology and hydrophilic properties, cultured iPSCs have shown increased chondrogenesis.[33] Altogether, it is evident that the nanomaterials in combination with iPSCs have potential for inducing chondrogenesis within the damaged tissues, thereby holding a promise for cartilage repair and regeneration.

Neural tissue engineering is an encouraging method for the regeneration of damaged nerves.[34] Studies have shown that iPSCs have been used in combination with nanoscale biomaterials for neural regeneration for a range of diseases and disorders such as parkinson's, alzheimer's, huntington's, spinal cord injury and traumatic brain injury.[35] For instance, Wang et al. (2011) have reported that iPSCs-derived multipotent neural crest stem cells (NCSC) seeded onto nanofibrous tubular scaffold nerve conduits can be used as a bridge for transected sciatic nerves in rat model.[36] The nanofibrous tubular scaffold has been made with PCL, poly(propylene glycol) and sodium acetate-based longitudinally aligned nanofibers. To further strengthen the scaffold, outer layers of random nanofibers were deposited onto the aligned fibres. The electrophysiological analysis demonstrated that NCSC-engrafted nerve conduits have an accelerated regeneration potential for sciatic nerves. NCSC seeded conduits transplantation has the ability to promote axonal myelination and can further support their differentiation into schwann cells. Furthermore, the differentiated NCSCs were found to be integrated into the myelin sheath around the axons. Interestingly, in a one year follow-up study for the *in vivo* implantation of NCSC seeded nerve conduits, no teratoma formation was revealed for upto one year, also a fully controlled microenvironment was formed for the differentiation of iPSCs-derived NCSC, thus emphasizing on the potential of iPSCs seeded nanomaterial-based scaffolds for nerve regeneration.[36] Scaffold properties can also modulate the behaviour of seeded iPSCs,[37] thereby emphasizing on the need to modulate scaffold properties compatible to the seeded cells. All these experimental examples, and other studies, clearly indicate that the nanomaterials loaded with iPSCs have great potential in regeneration of nerve cells for the treatment of neuronal diseases.

4. NANOMATERIALS FOR IMAGING AND TRACKING OF iPSCs

The iPSCs-based therapies offer significant potential in regenerative medicine. However, the knowledge regarding the *in vivo* kinetics of the transplanted iPSCs is still unclear, in particular how to monitor the cell imaging and trafficking in the living system. Nanotechnology provides an opportunity of developing a non-invasive method to repetitively monitor transplanted iPSCs *in vivo* and identify successful or unsuccessful engraftment outcomes. The use of superparamagnetic iron oxide (SPIO) nanoparticle for labeling the transplanted stem cells in magnetic resonance (MR) imaging, have been proven as an effective way for *in vivo* tracking of stem cells due to the near microscopic anatomical resolution, a longer blood half-life that permits longitudinal imaging and the high sensitivity for cell detection provided by MR imaging of SPIO nanoparticles. For instance, Castaneda et al. (2011) have reported a protocol for labeling iPSCs with ferumoxytol nanoparticles (17–30 nm), a FDA-approved ultrasmall superparamagnetic iron oxide (USPIO) composed of a non-stoichiometric magnetite core

surrounded by a polyglucose sorbitol carboxymethylether coat.[38] The results of this study have confirmed the technique as an efficient labeling system for iPSCs that leads to significant MR signal effects of labeled cells on MR images.[38] Similarly, Ruan et al. (2011) have also demonstrated the preparation and labeling of human-derived iPSCs using nanoparticles.[13] The authors labeled their iPSCs with fluorescent magnetic nanoparticles, which showed strong fluorescent and magnetic signals in MR imaging.[13] Interestingly, the long-term labeling and longitudinal imaging is attributed to the lysosomal storage of the iron oxide nanoparticles within the cells.[39] This technique can be further employed for the non-invasive monitoring of iPSCs-based therapies in pre-clinical and clinical settings. In relation to the effect of SPIO-labeled stem cells differentiation capacity, studies have demonstrated an SPIO dose dependent effect on differentiation capacity and SPIO doses do not impair differentiation potential of the stem cells.[40] Direct visualization of the transplanted iPSCs would allow for a better understanding of the factors that promote or impair successful iPSCs transplantations. Besides the advancements in the field, long term labeling do have some challenges such as dilution effect of the contrast agent over time and difficulty in identifying the differences in long-term signal kinetics of viable and dead cells which should be overcome in future. In over all, nanotechnology-assisted imaging and tracking of iPSCs is a milestone in bioimaging technology, which opens a new avenue for further research and development of iPSCs therapy.

5. NANOMATERIALS FOR GENETIC MODIFICATION OF iPSCs

Though iPSCs have provided an attractive alternate source to human ESCs, iPSCs are not yet considered as an ideal stem cell source, due to their limitations associated with the reprogramming process and their potential tumorigenic behavior. To provide a solution to this problem, many methods are being explored to generate iPSCs using nanotechnological principles and methods. In a recent approach, Lee et al. (2011) have reported an iPSCs generation method using non-viral magnetic nanoparticle-based transfection.[41] Biodegradable cationic polymer polyethyleneimine (PEI)-coated super paramagnetic nanoparticles were complexes to plasmid DNAs which comprised each of the four iPSCs factor genes.[41] The complex was exposed to the magnetic forces that guide gene vectors for all nucleic acid transfection toward normal mouse embryonic fibroblasts. Transfection was followed by nanofection-mediated iPSCs exhibiting ESCs-like characteristics. The results confirmed that non-viral magnet-based nanofection of iPSCs genes can result upto three-fold higher efficiencies of exogenous DNA-free safe iPSCs line production.[41] Similarly as already mentioned, Ruan et al. (2011) reported an efficient method of preparation of iPSCs for long-term tracing and imaging by co-transfecting four transcription factor genes (Oct4, Sox2, LIN28 & Nanog) and packing plasmids (PSPAX2 & PMD2.G) into 293T cells using generation 5.0 polyamidoamine dendrimer-modified magnetic nanoparticles (dMNPs) as a delivery system.[13] The results confirmed efficient delivery of all vectors into 293T cells through dMNPs. Despite the prolonged regulatory process required to enter the clinical arena, this type of nanomaterial-based delivery systems might offer advantages by increasing the rate of gene delivering into the cells and provide spatial and temporal control of the desired gene. The research work is in progress on this direction.

6. NANOMATERIALS FOR MANIPULATING MICROENVIRONMENT OF iPSCs

Controlling the fate and function of iPSCs *in vivo* is of paramount importance for the success of tissue engineering and regeneration. Many researchers worldwide are endeavoring to unveil the mechanisms by which the microenvironment (both physical and chemical cues) affects the lineage-commitment, as well as phenotype and function of iPSCs. Extrinsic cues from the native microenvironment of cells have been recently elucidated and included activation of different cellular pathways, growth factor binding and composition of the ECM. Recently, nanomaterials have been developed with the intention to successfully mimic or even bypass the effect of biological molecules in the iPSCs microenvironment. This is because cells in the human body live in a complex mixture of pores, ridges and components of micro and nano-featured environment, which are all, play a vital role in facilitating cell-matrix interactions and cell–cell communications upon implantation of the tissue graft. Many efforts have been directed at the construction of scaffolds for mimicking the natural ECM of the iPSCs.[42] It has been revealed that the ECM itself through its nanoscale geometry and interactions with cellular receptors can modulate the shape and therefore the gene expression of the cells. Therefore, nanomaterials can be found helpful in mimicking the iPSCs ECM through a variety of nanotopographies. Cell responses modulated by nanotopography include alignment, survival, motility, proliferation and differentiation. For instance, human iPSCs seeded onto nanostructured silicon substrates responded by elongating and aligning along the grating axis and expressed neuronal markers, whereas the same cells cultured on flat substrates spread randomly and conserved their pluripotent properties.[20] Similarly, it was found that iPSCs cultured onto an array of carbon nanotubes conjugated with ECM proteins to determine the pluripotent stem cell behavioral response, the nanotopographical array supported the undifferentiated iPSCs growth as well as self-renewal and also expressed pluripotency markers.[43] However, it was inferred that rough surfaces promote pluripotent stem cell adhesion with a more

compact morphology, but opposing to this, studies have proved evidence for whether or not nano-rough surfaces can maintain pluripotency and an undifferentiated state or induce spontaneous differentiation.[44] All these experimental examples, and other studies, have clearly demonstrated the impact of nanotechnology in the research and development of iPSCs.

7. CONCLUSION

The iPSCs are one of the attractive cell sources for tissue engineering and regenerative medicine. Nanotechnology has potential to develop various forms of nano-featured microenvironments to support the culture and growth of iPSCs specific to each application. Extensive studies have been carried out around the world to understand how the nano-features help to regulate or control the functional properties of iPSCs in engineering tissue and organs. As of today, nanotechnology offers the possibility to manufacture novel scaffolds that could modulate iPSCs fate and function. Despite numerous technological advances in the derivation of human iPSCs and their biomedical applications, relatively little is known about their interaction with nanoscale microenvironment which is very essential to develop physiologically functional engineered tissues and organs. The field of iPSCs nanotechnology is still in its infancy stage. Keeping these points in mind, future research may aim for converging nanotechnology, iPSCs biology and regenerative medicine in order to mimic the physiological complexity of the iPSCs microenvironment/niche and ultimately provide the multitude of required cell types for clinical therapies in humans.

Acknowledgments: This work was supported by CSCR. The author, Deepti Rana, would like to thank CSCR for the award of research fellowship.

References and Notes

1. D. Rana, T. S. Sampath Kumar, and M. Ramalingam, *J. Biomater. Tissue Eng.* 4, 507 (**2014**).
2. C. Bock, E. Kiskinis, G. Verstappen, H. Gu, G. Boulting, Z. D. Smith, M. Ziller, G. F. Croft, M. W. Amoroso, D. H. Oakley, A. Gnirke, K. Eggan, and A. Meissner, *Cell* 144, 439 (**2011**).
3. E. Hoveizi, S. Ebrahimi-Barough, S. Tavakol, and M. Nabiuni, *J. Biomed Mater. Res. A* (**2015**), Doi:10.1002/jbm.a.35420.
4. J. Liu, H. Nie, Z. Xu, X. Niu, S. Guo, J. Yin, F. Guo, G. Li, Y. Wang, and C. Zhang, *PLoS One* 9, e111566 (**2014**).
5. L. Chen, R. Qiu, and L. Li, *J. Biomed. Nanotechnol.* 10, 3431 (**2014**).
6. C. Du, K. Narayanan, M. F. Leong, and A. C. A. Wan, *Biomaterials* 35, 6006 (**2014**).
7. I. Ilie, R. Ilie, T. Mocan, D. Bartos, and L. Mocan, *International Journal of Nanomedicine* 7, 2211 (**2012**).
8. M. D. Bethesda, National Institutes of Health, U. S. Department of Health and Human Services, *Stem Cell Information* http://stemcells.nih.gov/info/basics/pages/basics10.aspx. (**2015**).
9. K. Takahashi and S. Yamanaka, *Cell* 126, 663 (**2006**).
10. J. Yu, M. A. Vodyanik, K. Smuga-Otto, J. Antosiewicz-Bourget, J. L. Frane, S. Tian, J. Nie, G. A. Jonsdottir, V. Ruotti, R. Stewart, I. I. Slukvin, and J. A. Thomson, *Science* 318, 1917 (**2007**).
11. Y. Wang, *New J. Sci.* 756240, 22 (**2014**).
12. M. Mimeault, R. Hauke, and S. K. Batra, *Clin Pharmacol. Ther.* 82, 252 (**2007**).
13. J. Ruan, J. Shen, Z. Wang, J. Ji, H. Song, K. Wang, B. Liu, J. Li, and D. Cui, *Int. J. Nanomedicine* 6, 425 (**2011**).
14. J. Holy, E. Perkins, and X. Yu, *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2009, 6022 (**2009**).
15. T. Kito, R. Shibata, M. Ishii, H. Suzuki, T. Himeno, Y. Kataoka, Y. Yamamura, T. Yamamoto, N. Nishio, S. Ito, Y. Numaguchi, T. Tanigawa, J. K. Yamashita, N. Ouchi, H. Honda, K. Isobe, and T. Murohara, *Sci. Rep.* 3, 1418 (**2013**).
16. T. Yamazoe, N. Shiraki, M. Toyoda, N. Kiyokawa, H. Okita, Y. Miyagawa, H. Akutsu, A. Umezawa, Y. Sasaki, K. Kume, and S. Kume, *J. Cell Sci.* 126, 5391 (**2013**).
17. N. S. Bhise, K. J. Wahlin, D. J. Zack, and J. J. Green, *Int. J. Nanomedicine* 8, 4641 (**2013**).
18. Y. Deng, X. Zhang, Y. Zhao, S. Liang, A. Xu, X. Gao, F. Deng, J. Fang, and S. Wei, *Carbohydr. Polym.* 101, 36 (**2014**).
19. A. Ardeshirylajimi, P. Dinarvand, E. Seyedjafari, L. Langroudi, F. J. Adegani, and M. Soleimani, *Cell Tissue Res.* 354, 849 (**2013**).
20. F. Pan, M. Zhang, G. Wu, Y. Lai, B. Greber, H. R. Schöler, and L. Chi, *Biomaterials* 34, 8131 (**2013**).
21. A. Ardeshirylajimi, S. Hosseinkhani, K. Parivar, P. Yaghmaie, and M. Soleimani, *Mol. Biol. Rep.* 40, 4287 (**2013**).
22. K. Gao, L. Li, L. He, K. Hinkle, Y. Wu, J. Ma, L. Chang, X. Zhao, D. G. Perez, S. Eckardt, J. McLaughlin, B. Liu, D. F. Farson, and L. J. Lee, *Small* 10, 1015 (**2014**).
23. E. J. Seo, I. H. Jang, E. K. Do, H. C. Cheon, S. C. Heo, Y. W. Kwon, G. O. Jeong, B. R. Kim, and J. H. Kim, *PLoS One* 8, e76875 (**2013**).
24. M. Khan, K. Narayanan, H. Lu, Y. Choo, C. Du, N. Wiradharma, Y. Y. Yang, and A. C. A. Wan, *Biomaterials* 34, 5336 (**2013**).
25. W. Chen, P. Tsai, Y. Hung, S. H. Chiou, and C. Y. Mou, *ACS Nano* 7, 8423 (**2013**).
26. M. H. Kim and M. Kino-Oka, *Biomaterials* 35, 5670 (**2014**).
27. N. K. Mohtaram, J. Ko, C. King, L. Sun, N. Muller, M. B. Jun, and S. M. Willerth, *J. Biomed. Mater Res. A* (**2014**), Doi:10.1002/jbm.a.35392.
28. F. D'Angelo, I. Armentano, I. Cacciotti, R. Tiribuzi, M. Quattrocelli, C. Del Gaudio, E. Fortunati, E. Saino, A. Caraffa, C. G. Cerulli, L. Visai, J. M. Kenny, M. Sampaolesi, A. Bianco, S. Martino, and A. Orlacchio, *Biomacromolecules* 13, 1350 (**2012**).
29. J. Wang, L. Wang, M. Yang, Y. Zhu, A. Tomsia, and C. Mao, *Nano Lett.* 14, 6850 (**2014**).
30. C. Chung and J. Burdick, *Adv. Drug Deliv. Rev.* 60, 243 (**2008**).
31. M. Demoor, D. Ollitrault, T. Gomez-Leduc, M. Bouyoucef, M. Hervieu, H. Fabre, J. Lafont, J. M. Denoix, F. Audigié, F. Mallein-Gerin, F. Legendre, and P. Galera, *Biochim. Biophys. Acta* 1840, 2414 (**2014**).
32. K. Tur, *Turk J. Rheumatol.* 24, 206 (**2009**).
33. J. Liu, H. Nie, Z. Xu, X. Niu, S. Guo, J. Yin, F. Guo, G. Li, Y. Wang, and C. Zhang, *PLoS One* 9, e111566 (**2014**).
34. C. E. Schmidt and J. B. Leach, *Annu. Rev. Biomed. Eng.* 5, 293 (**2003**).
35. S. M. Willerth, *Stem Cell Res. Ther.* 2, 17 (**2011**).
36. A. Wang, Z. Tang, I. H. Park, Y. Zhu, S. Patel, G. Q. Daley, and S. Li, *Biomaterials* 32, 5023 (**2011**).
37. F. Khayyatan, S. Nemati, S. Kiani, S. Hojjati Emami, and H. Baharvand, *Cell J.* 16, 53 (**2014**).
38. R. T. Castaneda, A. Khurana, R. Khan, and H. E. Daldrup-Link, *Journal of Visualized Experiments* 57, e3482 (**2011**).
39. S. Metz, G. Bonaterra, M. Rudelius, M. Settles, E. J. Rummeny, and H. E. Daldrup-Link, *Eur. Radiol.* 14, 1851 (**2004**).

40. T. D. Henning, E. J. Sutton, A. Kim, D. Golovko, A. Horvai, L. Ackerman, B. Sennino, D. McDonald, J. Lotz, and H. E. Daldrup-Link, *Contrast Media Mol. Imaging* 4, 165 **(2009)**.
41. C. H. Lee, J. H. Kim, H. J. Lee, K. Jeon, H. Lim, Choi, H. Y. Choi, E.-R. Lee, S. H. Park, J.-Y. Park, S. Hong, S. Kim, and S.-G. Cho, *Biomaterials* 32, 6683 **(2011)**.
42. R. Xu, M. B. Taskin, M. Rubert, D. Seliktar, F. Besenbacher, and M. Chen, *Sci. Rep.* 5, 8480 **(2015)**.
43. M. V. Pryzhkova, I. Aria, Q. Cheng, G. M. Harris, X. Zan, M. Gharib, and E. Jabbarzadeh, *Biomaterials* 35, 5098 **(2014)**.
44. K. Rutledge and E. Jabbarzadeh, *J. Nanomed. Nanotechnol.* 5, 217 **(2014)**.

Received: 7 December 2014. Accepted: 12 January 2015.