Application of Nanomaterials in 3 Dimensional Stem Cell Culture

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Abstract

Petri dish cultured cells have for long provided scientists an aperture to understanding cell's behavior both in normal and disease state as well as in vitro and in vivo. But recent advances have brought to light how the architecture and composite nature of the immediate environment within which the cell is proliferated can profoundly influence its phenotypic features and functions, thus making obvious, limitations of the conventional 2 dimensional cell culture despite it cost effectiveness. Fortunately, the transition to 3 dimensional cell culture has occurred concurrently with expanded knowledge of nanoscience and materials thereby lending significant impetus for innovative researches. This review is focused on application of nanoparticles in 3D stem cell breeding, recent trends and developments in medical sciences for improved drug delivery and treatment approaches to some human diseases. We also reviewed prevailing challenges and concerns of nano-toxicity as it continues to impede and delay clinical applications as well the ongoing concerted and multidisciplinary efforts to overcoming them.

Graphical Abstract

-Petri dish cultured cells have for long provided scientists an aperture to understanding cell's behavior both in normal and pathological state as well as in vitro and in vivo. But recent advances have brought to light how the architecture and composite nature of the immediate environment within which the cell is proliferated can profoundly influence its phenotypic features and functions, thus making obvious, limitations of the conventional 2 dimensional cell culture despite it cost effectiveness. - Fortunately, the transition to 3 dimensional cell culture has occurred concurrently with expanded knowledge of nanoscience and materials thereby lending significant impetus for innovative researches.

Keywords: Stem cell, Nanoparticles, Nanomedicine

Introduction

Scientists and researchers in recent decades, have intensified attention to studies relating to the field of nanoscience and its vast range of applications. Publications of results and findings from these studies have revealed great potentials and benefits for overall improvement in quality of life or human survival thereby making these endeavors even more promising and attractive, hence the huge traction attributable to the multidisciplinary researches with nanoscale materials (Ferreira, Karp, Nobre, & Langer, 2008). Scientists however are still locked in debates on the future implication of nanotechnology given its manifest capabilities to transform many frontiers and breakthroughs that were hitherto considered a dead end (Khan, Saeed, & Khan, 2017). In its broad sense, nanotechnology has been defined as the manipulation of matter (atoms & molecules) with at least one of its dimensions within the range of 1 nm to 100 nm (Saini, Saini, & Sharma, 2010). Accordingly, nanomaterial or nanoparticle is defined as a material or particle either natural occurring or artificially fabricated whose external dimension, internal and/or surface structure falls within the nanoscale range of 1 nm to 100 nm. It is worthy to note that 1 nm is equivalent to 10^{-9} m (1 billionth of a meter), in essence, emphasis is on the size (Buzea, Pacheco, & Robbie, 2007; Deb, Griffith, Muinck, & Rafat, 2012; ISO, 2015).

Dimensionality of nanomaterials plays an important role in shaping its physical, chemical and biological characteristic behaviors. Consequently, nanomaterials have been categorized into 0D, 1D, 2D & 3D (Tiwari, Tiwari, & Kim, 2012). The impacts of utilization of nanomaterials is already being felt across various specializations. In healthcare for instance, nanomedicine or nano-biotechnology has found profound application in targeted drug delivery and therapeutics, gene therapy, regenerative medicine, stem cell research, diagnostic tools & surgical aids amongst several biomedical devices (Deb et al., 2012).

Dimensional Stem Cell Culture

Stem cells are the 'master cells' of tissues and organs of the body which undergo differentiation to serve mainly for restoration or replacement of worn out, injured or diseased cells of various tissues and organs such as heart, bone marrow, brain, muscle tissues, skin etc hence their adaptation for regenerative roles (Schöler, 2016; Z. Wang, Ruan, & Cui, 2009). There are two major types of stem cell in mammals: embryonic stem cells (ESC) which are somatic cells capable of differentiating to become part of all cell types found in tissues and organs of the body such as heart, nervous, immune system etc therefore have been termed as pluripotent (Mahla, 2016). ESC originates from the inner mass cells of a blastocyst (Schöler, 2016). Whereas adult stem cells on the other hand are

multipotent. This means that they can differentiate into more than one types of cells of the tissues and organs of the body but are more limited compared to ESC, given their confinement to the family of the parent tissue they are derived from (same lineage)(Takahashi & Yamanaka, 2006). They are also described as non-pluripotent but with aid of advanced technologies, they have successfully been induced via genetic engineering to become pluripotent cells thus called induced pluripotent cells (iPCs)(G. Liang & Zhang, 2013; Metcalf, 2007; Oswald et al., 2004; Yu et al., 2007).

Generally, cell culture has a long history in cell biology and played a vital role in our modern era detailed understanding of both theoretical and experimental knowledge of cell biology and disease studies. Cell in its native physiological environment (in vivo) exist in three dimensional (3D) form which allows it to interact freely with its surrounding. It has been reported that the 3D culture models closely mimic the cell natural microenvironment in tissues and organs than cells grown in the conventional in vitro 2D approaches. Although 2D models are easy and simple to execute, its major limitations were over activation of proliferation signals which is the direct result of cells adhesion to plastic petri dish and inadequate interface or interaction with other cells. Based on these inherent disadvantages of 2D culture system, the need for 3D model thrived basically for its advantages of presenting the growing cells an enabling environment to form patterned growth such as suspension, adherent or multi-cellular clusters (spheroids) occasioned by cell-cell, and cell-matrix interaction, nutrient and oxygen circulation, waste removal as well as migration as they would normally do in vivo(Zhao, Tan, Pastorin, & Ho, 2013).

3D culture system can be grouped into two broad types, namely; scaffold and scaffold-free type. Scaffold provides a form of support for the proliferating cells and has been shown to confer physiochemical influences on the cells propagated in it(D. Liang, Hsiao, & Chu, 2007; Seyedjafari, Soleimani, Ghaemi, & Sarbolouki, 2011). Fortunately, technological advancement has occurred concurrently with expanded knowledge of stem cell biology, pathology and nanomedicine thereby lending commendable impetus for state-of-the-art researches in diagnosis, vaccine development, targeted drug delivery designs as well as other applications.

Application of nanomaterial in 3D Stem Cells Culture

Utilization of nanomaterials in stem cell research have been demonstrated to be a veritable and useful tool for immense advances in therapeutic studies, targeted drug delivery and pharmacological interventions, further molecular understanding of human physiology as well as regenerative medicine in general(Deb et al., 2012).

Some of the established areas where nanomedicine is making encouraging headways especially in stem cell application are discussed below in brief.

Stem Cell Labelling

Quantum Dot (Qdot).

Quantum dot (Qdot) is a light emitting nanocrystal usually 2-10 nm in size. they are mostly made from group II –VI (such as CdSe, CdTe, CdS or ZnSe) or group III – V elements of the periodic table (Figure 1). It has recorded wide usage for long term stem cell labelling, monitoring and tracking cell of survival, location and differentiation. Qdots were first reported for bio-labeling in 1998(Bruchez, Moronne, Gin, Weiss, & Alivisatos, 1998; Chan & Nie, 1998). Due to its acknowledged photo stability and long term fluorescence intensity, Qdot have gained more acceptance over organic dyes in immuno-labelling (Lei et al., 2007; Lin et al., 2007). Furthermore, it has larger surface area than conventional chromophores thereby making them suitable for conjugation with bio-recognition molecules such nucleic acid, peptides, antibodies or small-molecule ligands. For instance, Qdot is particularly suitable for labelling of certain protein especially during investigations of heart tissue injury to eliminate auto-fluorescence(Laflamme & Murry, 2005). Similarly, colloidal quantum dots have found a unique application as contrast agent for cell imaging both in vitro and in vivo (figure 2).

Superparamagnetic Iron Oxide (SPIO)

SPIO is a magnetic nanoparticle consisting of an inner iron oxide (Fe_3O_4) core with a dextran or carboxydextran coated exterior surface. The surface coating material guarantees its solubility in aqueous environment and avoids aggregation of nanoparticles(Reimer & Balzer, 2003; Y.-X. J. Wang, Hussain, & Krestin, 2001). SPIO is being used to improve the contrast of magnetic resonance imaging (MRI) of cellular targets. Similar to other nanoparticle labelling techniques, SPIO can be deployed by either surface attachment to stem cell or internalization by endocytosis or phagocytosis(Sykov & Jendelov, 2005) (figure 3). But Walczak et al suggested that electroporation is a much faster labelling method compared to SPIO (Walczak, Gilad, Kedziorek, Lin, & Bulte, 2005). However, it is important to note that surface labelling with SPIO is most suitable for in vitro studies compared to in vivo. This has been attributed to rapid detection of SPIO labelled cells and subsequent clearance by reticulo-endothelia apparatus(Lewin et al., 2000). Whereas cellular uptake of SPIO through endocytosis is well documented but may be aided with a transfection agent for in vivo cell tracking and yielding good magnetic resonance cell imaging as reported in human mesenchyme stem cell (hMSC)(Hsiao et al., 2007) , neural stem cell (NSC)(Guzman et al., 2007) labelled culture studies.

Nanoparticle Drug and Gene Delivery

Nanoparticles are being designed and used for gene delivery through the process of transfection. The major advantages of this approach over viral vector delivery methods is that the risk of viral infection, toxicity, mutagenesis and immunogenicity is completely eliminated or drastically reduced to tolerable levels(Glover, Lipps, & Jans, 2005; Pack, Hoffman, Pun, & Stayton, 2005).

Successful nanoparticle aided gene delivery have been reported in ESCs with apatite nanoparticles which were coated with fibronectin and E-cadherin with a significant 3 fold higher gene expression(Kutsuzawa, Akaike, & Chowdhury, 2008; Kutsuzawa et al., 2006). Also a similar successful delivery was achieved in mESC with nanoscale-constructed cell substratum cultured on a silicon nanowire array and interestingly cell viability was not impacted(Kim, Ng, Kunitake, Conklin, & Yang, 2007). Furthermore, Bianco et al reported use of carbon nanotubes (CNT) for delivery of drugs, other essential biomolecules such as nucleic acid and proteins into mammalian cells in vitro prior to scaffold seeding and culturing. But the prospect of carbon nanotubes utilization in stem cell research is being explored, however most recent studies and surveillance work are been focused at accessing it cytotoxicity(Ferreira et al., 2008).

Another promising revelation for nanoparticle application is its potentials for treatment of atherosclerotic plaques. A recent novel research demonstrated that modified gold nanoparticles delivered with adult stem cells were used to clear atherosclerosis plaques in the arteries of pig and also facilitated regeneration of the arteries(Deb et al., 2012). It further stated that the combination of nanoparticles with stem cells yielded better effect compared to nanoparticles alone hence underscoring the need for more exploration of the potentials of nanomaterials in stem cell research approaches. In a related development, 3D cell culture (spheroid) has been reportedly used as a model to examine nanoparticle penetration or uptake in solid avascular tumors of lungs, breast liver etc. The study employed nanoshell coatings made of poly ethylene glycol and phosphatidylcholine to conceal silica and citrate linked gold nanoparticles from immune detection thereby reducing immune triggered reactions and system wide toxicity. Although, 3D culture model does not possess all the features of the tumor microenvironment, the result revealed valuable basis for understanding the behavior of nanoparticles and possible utilization for cancer therapy such as targeted drug delivery or thermal (England, Christopher G., et al.,2013).

Tissue Engineering

Nanostructured biomaterials for both hard (bone) and soft (cartilages, connective) tissue engineering has being one of the focal hotspot for numerous researches in nanomedicine

and regenerative medicine. Due to the nature of these tissues, designs of enabling ESM or scaffold must take into account their special requirements such as structural support, high porosity (for cellular proliferation and diffusion) , and biodegradability(Karageorgiou & Kaplan, 2005; Mora, Valenti, García, & Giacomelli, 2010; Rezwan, Chen, Blaker, & Boccaccini, 2006). Interestingly, suitable ESM or scaffold is one of the primary requirements for 3D cell culture and bio-printing. In this regards carbon nanotubes (CNTs) have been extensively used to fabricate various scaffolds with varying percentages of nano-composite materials. Also scaffold that would allow and promote angiogenesis is another major factor for consideration(Tabata, 2009). As reported by Jell et al, poly ether urethane reinforced CNTs demonstrated significant results for vascularized bone tissue formation(Jell et al., 2008). Similar successes has been recorded in cartilage tissue engineering studies with CNTs in the presence of electrical stimulation(Iwasa, Engebretsen, Shima, & Ochi, 2009; Khang et al., 2007; MacGinitie, Gluzband, & Grodzinsky, 1994). In the case of connecting tissue such as the skin, cell cultured on a 3D nanofibrous composite scaffold yielded favorable results leading to the conclusion of its potential deployment for soft tissue regeneration and repair(Meng et al., 2009) (Figure 3).

3D nano-scaffold development

Scientist have successfully used nano-fabrication technologies to design 3D scaffold to stem cell proliferation. These biodegradable scaffolds closely mimic the cell's natural micro environment thus allows them to grow within it while depositing their own matrix as the scaffold is gradually degraded. Using a peptide nanofiber scaffold, a group of researchers succeeded in synthesizing 18 different peptides with mouse neural stem cells (mNSCs)(Z. Wang et al., 2009). A similar approach was adopted to study cartilage repair with electrospun, porous 3D nanofiber scaffold(Cui et al., 2010). Nano-fibrous structured scaffold have been shown to favorably support bone tissue (osteoblast, osteoclast, and fibroblast) proliferation and differentiation in 3D cell cultures for possible implantation(Teixeira, Abrams, Bertics, Murphy, & Nealey, 2003; Thorvaldsson, Stenhamre, Gatenholm, & Walkenström, 2008). In a recent report on breast cancer, it was revealed that 3D nanofiber scaffold exerted significant influence on CD44+ /CD24- by limiting their migration and invasion tendencies thus further highlighting the importance of cell-extracellular matrix interaction in stem cell proliferation which could be exploited for designing therapeutic interventions with better efficacies(Rijal & Li, 2016).

Clinical Applications of some Nanomaterials.

Allergic Asthma Treatment

A number of nanomaterials have been deployed for clinical treatment of certain illness which are hard to reach targets with conventional therapeutics. Chitosan nanosphere has been shown as a viable alternative for allergic asthma treatment compared the traditional drugs such as theophylline, methotrexate, cyclosporine, azathioprine with established limitations and side effects(Kumar et al., 2003; Lim et al., 2001). Chitosan is a derivative of chitin developed through N-deacetylation to form a positively charged biopolymer with properties such as biodegradability, biocompatibility, minimal toxicity, and low immunogenicity(T.-H. Kim et al., 2007). It is also reported to form stable DNA complexes called polyplexes (see fig. 1) and efficient intracellular gene delivery associated to its mucosa adhesive and poly-cationic nature(Okamoto et al., 2003).

Application in Cardiovascular Diseases Therapy

Nanocomposite fabrications have been found to demonstrate potentials to meet the delicate requirements for heart tissue regeneration, mechanical forte and physiological suitability such as anti-platelet properties, and hematological compatibility, hence hold promising chances for application in treatment of heart related diseases(Chen et al., 2010). In the area of heart patches, recent researches have shown that nanocomposite constructions of zien protein fused into electrospun PSG resulted in better water uptake and rigidity of the cardiovascular patch(Danielsen, Cao, Roursgaard, Møller, & Loft, 2015; Savi et al., 2014; Smyth et al., 2015). A similar improvement in vascularization was reported in a fabrication involving a decellularized heart tissue matrix and silk material incorporation(Savi et al., 2014). Furthermore carbon nanofiber reinforced with poly lactic-co-glycolic acid (PLGA) was reported to have enabled cardiomyocyte differentiation, migration and matrix attachment in culture environment making it another prospective heart patch option as a way of repairing and regenerating tissues after myocardial infarction(Stout, Basu, & Webster, 2011). Similarly a 3D culture of heart tissues using a scaffold fabricated from gold nanoparticles, ploy fibers of polycaprolactone and gelatin demonstrated amplified contraction and significant cardiac activities suggesting it potentials suitability for use as a cardiac patch and regenerative interventions(Shevach, Maoz, Feiner, Shapira, & Dvir, 2013). Likewise, nanoparticles fashioned into 3D scaffold have been used to attempt making artificial heart valves. An example of these was a valve fabricated from a nanomaterial called polyhedral-oligomeric-silsesquioxanes (POSS-PCU) strengthened with polyurethane and polycarbonate fragments reportedly displayed encouraging properties as a workable heart valve(Kidane et al., 2009). These Nano composites and nanoparticles have shown considerable opportunities for utilization in treatment of cardiac diseases but more studies

are required to address concerns of their long term effectiveness and improve overall performance(Jiang, Rutherford, Vuong, & Liu, 2017).

Toxicity of Nanoparticles

Although nanomaterial application in stem cell studies have revealed promising results, issues relating to its biocompatibility and toxicity has failed to achieve a consensus view. A number of studies have revealed conflicting conclusions about the cytotoxicity, neurotoxicity and genotoxicity of nanoparticles.

Studies conducted by Shah and Chakraborty stated that no adverse effect was noticed in the overall differentiation and morphological features of ESC cultured with qdots(Chakraborty et al., 2007; Shah, Clark, Moiola, Strosio, & Mao, 2007), but another study reported some abnormalities in proliferation pattern of the stem cell. However, some researchers have projected that the cytotoxicity of qdots may have resulted from its cellular degradation. Oxidative degradation of qdots produces Cd²⁺ ion which is known to cause cell poisoning through binding to sulfhydryl group of mitochondria(Derfus, Chan, & Bhatia, 2004; Hsieh, Wang, Hung, Chen, & Wang, 2006; Seleverstov et al., 2006). Furthermore, Dubertret *et al* also reported collection of qdots in nucleus of cells(Dubertret et al., 2002).

In the case of SPIO nanoparticle, reports of extensive investigations of cultured human mesenchyma stem cell (hMSC), mouse ESC, did not establish any cytotoxic effect following internalization of the nanoparticle by stem cells(Arai et al., 2006; Hsiao et al., 2007; Lu et al., 2007; Stroh et al., 2005). This can be attributed to the fact that it is composed mainly of biodegradable iron which can be recycled and used by other biochemical pathways(Hoepken, Korten, Robinson, & Dringen, 2004). However a study conducted by Bulte et al, in 2004 stated Fe from SPIO disrupted differentiation of hMSC(Bulte, Kraitchman, Mackay, & Pittenger, 2004) but Arbab *et al* in a later study attributed it to transfection agents and not SPIO nanoparticle(Arbab et al., 2004).

Cytotoxicity of carbon nanotubes in hESCs, mESCs and hMSCs have been assessed in a number of studies(Corsi, Chellat, Yahia, & Fernandes, 2003; Ferreira et al., 2008; Tran, Ota, Jacobson, Patton, & Chan, 2007). It was unanimously reported that carbon nanotubes toxicity maybe a function of it concentration , exterior coating as well as its physical features such as size and shape(Magrez et al., 2006). In the same manner, genotoxicity of multi-walled carbon nanotubes (MWNTs) was highlighted for it induction of apoptosis in mMSCs , tumor gene suppression abilities, mutagenesis(Zhu, Chang, Dai, & Hong, 2007) as well as generation of reactive oxygen species(Joshi et al., 2008). In view of this issues further investigation would be necessary to establish it toxicity mechanism and hence determine it biocompatibility in stem cell works.

Conclusion

The synergy between cell culture and nanoparticle is rapidly evolving with promising potentials for improved health care delivery. Nanomaterial applications in 3D cultures appears to have dawn a new chapter in stem cell studies informing changes in approach to tissue engineering, drug and vaccine targeting, cellular imaging and tracking. Interestingly some of these findings are now being utilized for preventive and therapeutic interventions in areas of respiratory disorders, cancer and tumor treatment, tissue transplantations. Besides its physicochemical properties being employed for these therapeutic purposes, nanomaterials have also significantly advanced the fabrication of suitable 3D scaffolds or matrices for cell culturing especially the composite or reinforced scaffold which facilitates noticeable and distinctive topographies of cell proliferation on it as would naturally occur in vivo. On the basis of the proven low immunogenicity and toxicities of some nanoparticles, the future of nanomedicine is beaming very brightly. Worthy of note in this perspective is the payload capacity of these fabricated 3D nanostructures. Applicability for targeted delivery of therapeutic agents such as proteins, genes, engineered cells or tissues, attenuated microbial (viral) particles are bound to be more precise thereby enhancing their effectiveness on cancerous or tumor cells with assured minimal side effects to normal cells of other tissues and organs of the body as against the system wide side effects known of current cancer therapies. With this growing realization of the biological advantages of 3D cell culture, we thus can envisage the 2D approach embarking on a relegation journey to the obsolete realm in the near future.

Although significant progress has been made in advancing the prospect of nanoparticles in medicine, challenges of nano toxicity however have lingered over the years. This being one of the major concerns have hindered approval from relevant biosafety and ethical regulatory bodies therefore truncating the transition of many of these laudable achievements to clinical phase. Further studies are required to fully understand the neurotoxic impacts, possible biochemical complications of these materials over long periods with a view of addressing such side effects to pave the way for the much needed nanomedicine products for beneficiaries.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Contributions

SG: Planned the study, wrote the protocol, collected the data and drafted the manuscript and accepted the final draft; MPA and KK: Planned and designed the study, collected the data. OO, analyzed the data and critically revised the draft and finally approved the manuscript.

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Figure 1: Nanoparticles & scaffold for Stem Cell Research. This image adapted from(Ferreira et al., 2008) with copyright permission.

Figure 2: Cell labeling with quantum dots and illustration of quantum dot photostability, compared with the dye Alexa 488. In the upper panels, the nucleus is stained red with quantum dots and the actin fibers are stained green with the dye. In the lower panel, the labeling is reversed. This image adapted from (Alivisatos, Paul.,2004) with copyright permission.

Figure 3: Nanofiber-Assembly Scaffolds for Tissue Engineering. This image adapted from (Ferreira et al., 2008) with copyright permission.

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