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APPLICATION OF NANOMATERIALS IN MESENCHYMAL STEM CELL ENGINEERING

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Mesenchymal stem cells (MSCs) hold great potential for the treatment of many injuries, degenerative diseases and stem cell engineering. Stem cells are structurally suitable to make nanoparticles biocompatible and offer a clinically proven, versatile platform for the further enhancement of pharmacological efficacy. These include the development of advanced techniques to understand and control functions of micro environmental signals and novel methods to track and guide transplanted stem cells. Application of Ferridex, nanoACP, nanoHAP, magnetic nanoparticle, quantum dot, liposomal-ceramide and biomatrics based nanoparticle in mesenchymal stem cell research has been described in terms of their potentials, as diagnostic and therapeutic multimodality agents.

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1. Introduction

Stem cells are defined as clonogenic, self-renewing progenitor cells that can generate one or more specialized cell types. Embryonic stem cell (ESC) lines, established in human in 1998 [1], are derived from the inner cell mass of the blastocyst and are capable of generating all differentiated cell types in the body. To date, there are more than 300 human ESC lines, but only 22 human ESC lines are commercially available and registered with the NIH (http://stemcells.nih.gov/research/registry/) [2]. ESC cells are pluripotent that can differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. Pluripotency indicates ability to differentiate into any of the 200 different known cell types. On the other hand adult stem cells are still pluripotent, but their differentiation ability is restricted to the cell types of a particular tissue, being responsible for organ regeneration. Two general categories of reserve precursor cells exist within the body and are involved in the maintenance and repair of tissue in adults: a) lineage-committed progenitor cells, and b) lineage uncommitted pluripotent stem cells [3].Progenitor stem cells may be committed to one or more specific tissue lineages, which can be further classified into unipotent, bipotent, tripotent, or multipotent, respectively [3]. Each progenitor cell for a particular tissue lineage has a unique profile of cell surface cluster of differentiation (CD) markers [3]. Primitive stem cells within the bone marrow niche, haematopoietic stem cell, (HSC) possess functional versatility, which is termed trans-differentiation or stem cell plasticity [4]. Stem cell plasticity describes the ability of adult stem

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cells to cross lineage barriers and to adopt the expression profiles and functional phenotypes of cells unique to other tissues [5]. HSC, expressing markers of the hematopoietic lineage ($CD45^+$) and of hematopoietic stem cells ($CD34^+$, $CD133^+$,and $CD117^+$, Thy-1^{low}, but $CD10^-$, $CD14^-$, $CD15^-$, $CD16^-$, $CD19^-$, and $CD20^-$) [6-7], are capable of genomic reprogramming upon exposure to a novel environment and give rise to other tissues such as liver, cardiac muscle, or brain [8-9].

Bone marrow has been the primary source for two stem cell populations: the human hematopoietic stem cells (hHSCs) and the stromal mesenchymal stem cells (MSCs). Recent studies suggest that adipose aspirate is also a rich source of MSCs [10], but their property and application in vivo remains to be fully investigated. MSCs are a heterogeneous cell population that has been shown to differentiate into bone, cartilage, fat, and fibrous tissues. In ex vivo culturing conditions, differentiation of MSCs into specific cell types can be guided by applying appropriate growth factors or chemicals [11]. The HSCs in mice transplanted at the single cell level gave rise to lifelong hematopoiesis [7]. Recent in vivo evidence has demonstrated the therapeutic effect of implanted MSCs in tissue repair [12-13]. MSCs are able to populate injured tissues through either local implantation or via systemic delivery. For example, MSCs directly transplanted into damaged heart muscles, can generate new muscle cells, while intravenous introduction of MSCs can induce angiogenesis in the heart and enhance the proliferation of existing cardiac vasculature [14]. In addition to HSC, bone marrow also contains mesenchymal stem cells (MSCs), which can be differentiated into bone cartilage fat and connective tissues. MSCs have tremendous potential for the repair and regeneration of damaged tissues and organs by extensive proliferation [15]

Properties and functions of stem cells have been extensively studied in the development of organisms [16], cancer [17], wound healing [18-19], and regenerative medicine. In the latter, it has been investigated for tackling complex pathogenic conditions such as neurodegenerative diseases [20], hematopoietic impairment [21], and musculoskeletal degeneration [22-23]. In the development of organism, the single totipotent cell after fertilization divides and specializes into pluripotent cells, such as embryonic stem cells that are necessary for fetal development. The pluripotent cells then further specialize into multipotent cells that commit into lineages with tissue-specific functions. Cells have been successfully identified in or isolated from embryonic [24], fetal [25-26], or adult tissues [27] and demonstrated to have stem cell-like properties in vitro and in vivo. The maintenance, survival and activity of these stem cells are suggested to be dependent on the special micro-environmental niche [27].

Cell based therapeutics is one of the most rapidly growing fields in translational medicine. Blood transfusion and bone marrow transplantation are two typical examples of cell based therapeutics. Currently stem cells are becoming rising starts in cell based therapeutics for tissue engineering and cell replacement therapy because of their pluripotency and self renewal capability. There are limited attempts to evaluate potential desirable effects of nanoparticles; on MSCs. Application of nanoparticle in stem cell based therapeutics is still in the preclinical development stage. Stem cell-nanoparticle constructs present great opportunities in clinically proven, versatile platform for the further enhancement of pharmacological efficacy. This review article aims at providing the current status of nanoparticle use in stem cell therapeutics.

2. Nanoparticles

Nanoparticles are tiny materials (<1000 nm in size) have a very high surface area to volume ratio. This makes the particles very reactive or catalytic [28]. Nanoparticles are easier to pass through cell membranes in organisms and get interacted rapidly with biological systems [28]. Such properties make them very attractive for commercial and medical development. However, nanoparticles can act on living cells at the nano level resulting in biologically desirable effects. Recent advances in creating nanomaterials have created new opportunities in biomedical research and clinical applications [29]. Many new nanoassays have higher sensitivity, selectivity and throughput than conventional bioanalytical methods. Conversely, these assays will lead to low-cost, point-of-care devices for rapid diagnosis of pathogenic and genetic diseases (e.g., HIV and cancer) [30]. Recently, nanomaterials such as nanotubes, nanowires, fullerene derivatives (bucky balls), and quantum dots have received enormous attention in the creation of new types of analytical tools

for biotechnology and the life sciences. Moreover, Lee *et al.* highlight the recent applications of nanoparticles in stem cell research [31].

3. Nanoparticle mediated MSCs differentiation

(i). Feridex

Previous studies show hHSCs homing and engraftment was performed using genetic labels, radionuclides or membrane dyes *in vivo* [32]. Recently it was demonstrates that, long-term repopulating cells, labeled with native or dextran-coated superparamagnetic iron oxide nanoparticles using magnetic resonance imaging (MRI). In an experiment $CD34^{+}$cells of hHSCs population labeled with fluorophore, feridex (Fe[647] or Fe[750]) nanoparticle, under defined, clinically applicable, serum-free *ex vivo* condition. When transplanted into immiunodeficient NOD/SCID, β2M null mice. Fluorescence imaging and flow cytometry analysis of both the bone marrow and haematopoietic organs revealed presence of $Fe[750]^{+}CD34^{+}$ labeled cells for up to 3 weeks. Moreover, for precise quantization of the cell population that had sequestered the nanoparticles and post transplantation fate was shown by fluorescence-activated cell sorting. Feridex are inert, biocompatible nanoparticles that are eventually metabolized and enter into whole body iron metabolism pathways [33]. This technique provides a method by which investigators can track human stem cells from the marrow versus different tissues of immune-deficient mice. This has been extremely difficult in the past, because stem cells can alter their phenotype after engraftment. The use of fluorophore-labeled feridex nanoparticles offer an efficient and safe method to label both cycling and noncycling hHSCs, without toxicity as well as to evaluate the homing, localization, phenotype, and short-term engraftment capabilities of defined hHSC subsets.

(ii). NanoACP and nanoHAP

Amorphous calcium phosphates (ACP) and hydroxyapatite (HAP) are the most preferred source for graft materials in hard tissue engineering, due to being its light weight and chemical stability. Moreover, both are accepted as suitable biomedical materials due to its chemical and structural similarities to bone. In clinical applications, HAP is used in various forms like coatings, filling of bone gaps or especially for bone-related remedies in reconstructive orthopedic and dental surgery [34]. Chemical similarity of nHAP with natural bone leads to an excellent biocompatibility and biomimicry. HAP is also employed as bone tissue engineering scaffold material and coating material for some bio-inert metals such as titanium and zirconia.[35-36] Better supermicrostructural biomimicry and osteoconductivity can be achieved if nano-sized HAP (nHAP) is employed as biomaterial for MSCs applications. ACP possesses excellent biocompatibility and osteoconductive characteristics. Among all the ACP derivatives HAP considered as a model inorganic component of bone and tooth enamel due to its high stability and least solubility. Furthermore, ACP is treated as a precursor of biological apatite during bone formation due to its high solubility and easy bioavailability. It has been shown that osteoblasts and bone marrow mesenchymal stem cells (BMSCs) plays most important role in biological mineralization processes. However, the exact mechanism on the effect of ACP coatings on bone response is still not clear. *In vitro* studies reveal higher osteogenic differentiation on ACP substrates as compared to crystalline hydroxyapatite. Moreover, increased osteoblast adhesion on nanoACP compared to nanocrystalline HAP has been shown [37]. The size of the nanoamorphous calcium phosphate and the nanocrystalline HAP were ~13 and ~31 nm, respectively. Thus the size of HAP greatly affects the adhesion and proliferations of BMSCs and osteosarcoma cells. Furthermore, the effect of ACP and HAP on the adsorption, proliferation, and differentiation of BMSCs has been demonstrated, keeping particles of uniform size distribution, ~20 nm. Some interesting findings reported are as 1. inhibition of adhesion of BMSCs on NanoACP as compared to that on the nanoHAP phase. 2. proliferation of BMSCs increased on NanoHAP than NanoACP. 3. higher rate of differentiation of BMSCs to osteoblasts on Crystallized calcium phosphate (NanoHAP) phase than amorphous calcium phosphate (NanoACP) [37]. Therefore, being a

promising material for biomedical application, nHAP attracts much interest. Studies on its biological properties and potential applications of bone marrow-derived MSCs play an important role in regenerative medicine and cell based therapy, which are the similar to ESCs but are free of ethics argument and risk of tumorigeness.

(iii).Quantum dots

Quantum dots (QDs) are an emerging field of fluorescent probes for non-invasive *in vivo* multiplex bioimaging due to their extreme brightness and resistance to photobleaching. QDs are semiconductor nanocrystals that can be excited by a wide range of light, ranging from ultraviolet to near-infrared and emit light of different wavelengths, depend on their size and composition. QDs have broad excitation spectra and narrow emission spectra because it can be excited by a single wavelength and can emit light of different wavelengths. QDs have been used for tumor targeting and imaging, lymph node and vascular mapping, and cellular trafficking and many advantages over traditional organic dyes [38]. In stem cell therapy, monitoring of cell survival and location after transplantation is important for determining their efficacy. Studies revealed 72% positive cells was demonstrated after 24h of incubation in living mice transplanted with QDs labeled ES cells in mice. However, the percentage of positive cells dropped to 4% after 4 day. The decrease in positive cells indicates rapid division of ES cells (doubling time of 12–15 hours) or QD diffusion out of dividing cells over time. Furthermore, any adverse effects of QDs on the viability, morphology, self renewal, pluripotency or development of ES cells were not observed [39].

(iv).Liposomal-ceramide

Liposomes (~100 nm and larger) is mainly useful in solubilizing drugs and extending circulation times to favor higher uptake of drugs in blood in various experimental models. Recently, propagation of undifferentiated hESCs with nano liposomal ceramide mediated differential expression of neuroectodermal markers, nestin and Tuj1 has been shown [40]. Ceramide is a bioactive sphingolipid, which converts into pro mitogenic metabolites in presence of ceramide metabolizing enzymes. Moreover, liposomal ceramide can be used to maintain undifferentiated hES cells, free of feeder cells. hESCs treated with liposomal ceramide maintain their pluripotent state as demonstrated by *in vivo* and *in vitro* differentiation studies, without any chromosomal abnormalities. It was suggested that exposure to ceramide provides a viable strategy to prevent premature hES cell differentiation and to maintain pluripotent stem cell populations in the absence of feeder cells [40].

(v). Magnetic nanoparticle

Magnetic nano-particles (MNPs) possess unique magnetic properties and the ability to function at the cellular and molecular level of biological interactions making them an attractive platform as contrast agents for magnetic resonance imaging. Recently, application of quadruple magnetic flow sorter (QMS) mediated magnetic nanopartiles combined with antibody CD34**,** successfully enriches peripheral blood progenitor cells (PBPCs) in samples of whole blood [41]. PBPCs are known as CD34+ cells because they express a protein CD34, on their cell surfaces. Clinical trials have shown that PBPCs are more effective than bone marrow transplantation at restoring an individual's blood cells population following high-dose chemotherapy or radiation therapy. Magnetic nanoparticles have a significant impact on both clinical oncology and basic cancer research.

(vi). Electrospun nanofibers

Recently, artificially aligned nanometer features to mimic natural matrices have been revealed, a powerful influence of nanotopography over cellular behavior. Aligned nanofibers composed of hydroxybutyl chitosan, when electrospun to create a robust scaffold, a aligned cell

sheets. Size of fiber diameter was 436nm. When hMSCs cultured on these surfaces, alignment and elongation in both cell body and nucleus has been demonstrated [43]. In addition to morphological changes, topographical features induced expression of genes indicative of myogenic induction of hMSCs cultured in proliferative, non-differentiating medium. On the other hand thermal reversibility of the fibers allows for the dissolution of the polymer from the cell/scaffold construct without disruption of cytoskeletal structure and cell–cell interactions. Thus production of poymer free cell layers engineered using nanotopographical cues provides tremendous opportunities in tissue engineering and cell-based regenerative medicine. The production of polymer-free tissue constructs has gained much attention in recently, mainly in the form of cell sheet engineering.

(vii). Nanotopography

MSCs have ability to differentiate into all cell types, including neurons, cardiomyocytes, hepatocytes, islet cells, skeletal muscle cells, and endothelial cells [42]. Reports suggest that topography of extracellular microenvironment influence cellular attachment, migration, differentiation and production of new tissue [43]. Moreover, changes in cell shape and gene expression on nano topography surfaces for example differentiation of stem cells into neuron and muscle has been well documented in rat and human bone marrow stromal cells [44], when cultured on nano patterns with gratings of 350 nm line width in MSCs proliferation medium. Significant elongation in cell bodies and nuclei of the hMSC was shown. In addition, expression of neuronal marker such as microtubeule associated protein2 and β-Tubulin III was also detected [43]. Thus nanotopography with or without the presence of biochemical signals, plays an important role in regulating stem cell differentiation.

(viii).Nanobiomatrics

MSCs depend on a variety of exogenous factors like soluble growth factors, mechanical stimulation and matrix stiffness to induce differentiation. Since cell and nuclear shapes are intimately regulated by intracellular signaling cascades for gene expression [45]. Moreover, various tissues are comprised of highly organized layered structures such as myocardium, vascular wall and corneal epithelium. Therefore, the introduction of topographical cues to create an aligned cell sheet may have tremendous applications for regenerative medicine. These aligned nanofibrous scaffolds composed of a thermally reversible polymer hydroxybutyl chitosan, a multifunctional scaffold. Which provide topographical cues to induce cell alignment with potential to directly influence expression of gene [45]. Furthermore, bioactive materials, such as natural matrix components and growth factors, could also be incorporated into the fibers during fabrication to provide an active surface of the scaffold, to enhance cellular functions. Altogether, such type of multifunctional scaffold system could be useful in regenerative medicine.

4. Conclusions

However, the mechanisms underlying the nanomaterial mediated MSCs engineering are still not clear enough. Nanomaterials, offer significant promise and are worthy of further exploration in attempts to enhance the differentiation, maintenance and providing biological clues for hMSCs mediated diagnosis. Altogether, nanomaterials provide tremendous opportunities in stem cell engineering and cell-based regenerative medicine.

References

[1] Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Science **282**:1145 (1998).
[2] Vander BKE, Swijnenburg RJ, Cao F, Wu JC. Cell Cycle **5**:2748 (2006).
[3] Young HE, Black AC, Jr. Anat Rec A Discov Mol Cell Evol Biol **276**:75(2004).
[4] Kiel MJ, Morrison SJ. Immunity **25**:862 (2006).

[5] Moraleda JM, Blanquer M, Bleda P, et al. Transpl Immunol **17**:74 (2006).
[6] DeCoppi P, Bartsch G, Jr., Siddiqui MM, et al. Nat Biotechnol **25**:100 (2007).
[7] Shizuru JA, Negrin RS, Weissman IL. Annu Rev Med. **56**:509 (2005).
[8] Cerny J, Quesenberry PJ. J Cell Physiol **201**:1 (2004).
[9] Herzog EL, Chai L, Krause DS. Blood **102**:3483 (2003).
[10] Gimble J, Guilak F. Cytotherapy **5**:362 (2003).
[11] Pittenger MF, Mackay AM, Beck SC et al Science **284**:143 (1999).
[12] Mangi AA, Noiseux N, Kong D et al. Nat Med **9**:1195 (2003).
[13] Miyahara Y, Nagaya N, Kataoka M et al Nat Med **12**:459 (2006).
[14] Ryan JM, Barry FP, Murphy JM et al. J Inflamm (Lond) **2**:8 (2005)
[15] Le Blanc K, Ringden O. Curr Opin Immunol.**18**:586 (2006).
[16] Koestenbauer S, Zech NH, Juch H et al. Am J Reprod Immunol **55**:169 (2006).
[17] Reya T, Morrison SJ, Clarke MF et al. Nature **414**:105 (2001).
[18] Roh C, Lyle S. Pediatr Res **59**:100 (2006).
[19] Sell S. Wound Repair Regen **9**: 467 (2001).
[20] Levy YS, Stroomza M, Melamed E et al. J Mol Neurosci **24**:353 (2004).
[21] Ringden O, LeBlanc K. Apmis **113**:813 (2005).
[22] Tuan RS. Clin Orthop Relat Res **1**:105 (2004).
[23] Wagers AJ, Conboy IM. Cell **122**:659 (2005)
[24] Semb H. Apmis **113**:743 (2005).
[25] Korbling M, Robinson S, Estrov Z et al. Cytotherapy **7**:258 (2005).
[26] Shafritz DA, Oertel M, Menthena A et al. Hepatology **43**:89 (2006).
[27] Moore KA, Lemischka IR. Science **311**:1880 (2006).
[28] Mishra VK, Mohammad G, Mishra SK. Digest Journal of Nanomaterials and Biostructures **3:** 163 (2008).
[29] Alivisatos AP. Nat. Biotechnol **22:** 47–52 (2004).
[30] Rosi NL, Mirkin CA. Chem. Rev **105:** 1547–1562 (2005).
[31] Solanki A, Kim JD, Lee KB. Nanomedicine **3:** 567–578 (2008).
[32] Bonde J, Hess DA, Nolta JA. Curr Opin Hematol **11:**392–398 (2004).
[33] Dustin JM, Jesper B, David AH, Sarah AH, Ryan L, Ping Z, Michael HC, David PW, Jan AN. Stem cells **26**:517–524 (2008).
[34] Qinghong H, Zhou T, Yukan L, Jinhui T, Yurong C, Ming Z, Haihua P, Xurong X, Ruikang T. J. Mater. Chem **17**: 4690 (2007).
[35] Kim HW, Kim HE, Knowles JC. J Biomed Mater Res B Appl Biomater; **70**: 270 (2004).
[36] Kim HW, Kim HE, Salih V, Knowles JC. J Biomed Mater Res; **685**: 22 (2004).
[37] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Science **284**: 143 (1999).
[38] Shuan L, Xiaoyan X, Manish K, Patel R, Yao-Hung Y, Zongjin L, Feng C, Oliver G, Yan Z, Sanjiv SG, Jiang HR and Joseph CW. BMC Biotechnology **7**:67 (2007).
[39] Choy G, Choyke P, Libutti SK: Mol Imaging, **2:**303 (2003).
[40] Ugur S, Todd EF, Nurgul CS, Arati S, Gavin PR, Mark K, Kent V. Stem Cells and Development. Doi:10.1089/scd.2007.0271.
[41 Jing Y, Moore LR, Williams PS, Chalmers JJ, Farag SS, Bolwell B, Zborowski M. Biotechnol Bioeng **96**:1139 (2007).
[42] Fuchs E, Segre JA. Cell **100:**143 (2000).
[43] Evelyn KFY, Stella WP, Kam WL. Exp Cell Res. **313**: 1820 (2007).
[44] Woodbury D, Schwarz EJ, Prockop DJ, Black IB. J Neurosci Res **61**:364 (2000).
[45] Jiyoung MD, Kam WL. Adv Mater Deerfield **19**: 2775 (2007).