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REGULAR ARTICLE

Near-IR absorbing quantum dots might be usable for growth factor-based differentiation of stem cells

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Abstract For stem cell therapy of degenerative diseases, it is necessary to differentiate stem cells into the specific lineage. There are several growth factors which have been used for differentiation of stem cells. Some growth factors can dose-dependently induce differentiation of stem cells so that the increase of growth factor concentration results in production of the higher level of differentiated cells. However, due to the toxicity of some differentiation factors (e.g. retinoic acid), the lower dose of growth factors for the specific lineage differentiation of stem cells is desirable. This paper suggests a new approach in the field of controlled growth factor delivery system using semiconductor nanocrystals; known as quantum dots (QDs). This system contains polymeric microencapsulated growth factor which is conjugated to near infrared (NIR) absorbing QDs. The control release of growth factors from microcapsules in the culture plates can be achieved by irradiation. To modulate growth factor release in response to stem cells needs for differentiation, the intensity and period of irradiation will be controlled. Our hypothesis is based on the fact that QDs can absorb NIR energy and by excitation of electrons and then vibrational relaxation of them become heated when they were irradiated and then release growth factors. We believe that controlled growth factors delivery through the suggested system is an effective method to reduce the amount of growth factors required for differentiation of stem cells.

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Introduction

Stem cell therapy is a new approach to repair diseased tissues or organs [1]. However, to achieve therapeutic efficacy, differentiation of stem cells into the specific lineage prior to transplantation is often essential [2]. To differentiate stem cells *in vitro*, growth factors are used [2,3]. The current common method for differentiation of stem cells has been based on continuum concepts where the culture medium is supposed to be homogeneous and an enough amount of growth factors is assumed to be contained in the medium [4]. However, because of Brownian motion of growth factor in the medium, only a small amount reaches the cell receptors related to biological signal pathways [4]. Therefore, even if a huge amount of growth factor is added to culture medium to induce differentiation, only a small fraction would be involved in differentiation of stem cells. Therefore, the chance of growth factor binding to cultured stem cells should be maximized to enhance differentiation.

Moreover, the use of some of these growth factors presents a significant challenge due of their poor water solubility, short half-life and potentially toxic effects. One example is all-trans-retinoic acid (RA), a hydrophobic drug, which plays a fundamental role in the development of the central nervous system, stimulating outgrowth and migration of the neural crest [5]. RA is one of the most important signaling molecules that promote neuralization in embryos [6–8]. RA, which can bind to both RAR subtypes, is commonly employed to induce neuronal differentiation *in vitro*. RA induces a pan-neuronal differentiation and the cell population obtained after application of this differentiation factor is relatively heterogeneous [9,10]. RA applied to embryonic stem cells (ESCs) can induce concentration-dependent differentiation of neural cells. Okada et al. [11] tested the effects of different concentrations of RA on the neural differentiation of mouse (ESCs). Lower RA levels were found to induce neural progenitor cells from ESCs, indicated by the high protein expression of the neural precursor marker nestin and low expression of mature neuronal and glial markers beta-tubulin III and GFAP, respectively. In contrast, high levels of RA (2×10^{-6} M) decreased the expression of nestin while increasing beta-tubulin III and GFAP levels. These results are consistent with other studies demonstrating differentiation of neural progenitors at high RA concentrations [12,13]. However, RA is a strong teratogen and should be used at lower doses to prevent toxicity [7]. Therefore, a method that can reduce the amount of growth factors required for cell differentiation should be developed. In addition, RA is rapidly metabolized to inactive polar metabolites such as all-trans-hydroxyl RA and all-trans-4-oxo-RA. This rapid metabolism of RA is due to the induction of the cytochrome P450 by RA [14]. To overcome such problem and to increase the differentiation efficiency of growth factors, a new approach is growth factor delivery from the cell culture scaffolds. It has been shown that growth factor released from a cell culture scaffold could bind to receptors on the cultured stem cells much more efficiently than growth factors added to the culture medium. Growth factor delivery using a cell culture matrix could also potentially reduce the amount of growth factor required for stem cell differentiation [15,16]. Through this approach, it will be possible to have adequate optimized differentiated cells for stem cell therapy and regenerative medicine and overcome the problem of donor cell and tissue shortage.

Hypothesis

Conjugation of a growth factor such as RA to a synthetic cell culture scaffold offers a localized release system for growth factor. Immobilization of RA to the scaffolds results in very slow release. In this study, we hypothesize a system of microencapsulated growth factor conjugated to near infrared (NIR) absorbing quantum dot (QD), in which controlled release of growth factors from the polymeric microcapsules is triggered by NIR irradiation. The energy of NIR radiation is in the vibrational levels of atoms and when a QD absorbs NIR, the valence electron excited to the upper vibrational levels. Then during 10^{-10} to 10^{-13} second electrons come back to prior vibrational level and release excess energy in the heat form. Our hypothesis is based on the fact that QDs can absorb NIR energy and by excitation of electrons and then vibrational relaxation of them become heated when they were irradiated and then release growth factors. This system can externally modulate release of growth factors like RA and EGF in response to stem cells needs for differentiation by control of the intensity and period of irradiation. We think by using this system and controlling of time and amount of release, the lower levels of growth factors will be required (at physiological level) to induce lineage-specific differentiation of stem cells. The selectivity of release is depending on the type of differentiation factor, intensity and period of NIR irradiation and the other components of scaffold.

Evaluation of the hypothesis

To test this hypothesis, NIR-QDs (Cadmium-Selenide); semiconductor nanocrystals with size-dependent absorption and emission, will be used. The surface chemistry of QD can regulate the solubility of QD, for example by addition of a layer of amphiphilic molecules as well as can functionalize QD [3]. Since RA is a hydrophobic growth factor, at first step it will be required to carboxylate QD. Then, crosslinking agents such as 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and sulfonated N-hydroxysuccinimide (sulfo-NHS) will be used. In order to conjugate QDs and RA, phenylene diamine (PDA) will be added to the activated QDs and RA solutions (Fig. 1).

Microencapsulation of QD-RA will be performed by double emulsion in solution of PCL and PLGA. After washing with deionized water, the microcapsules will be lyophilized. The morphology of microspheres will be examined with scanning electron microscopy. To avoid enzymatic degradation of the scaffold with matrix metalloproteinases secreted by stem cells, it is necessary to use the synthetic scaffold. The cell culture plates will be coated with PLGA containing QD-RA microspheres. The kinetics of growth factors release following irradiation will be determined with HPLC. ESCs will be cultured on PLGA scaffold loaded with the definite amount of QD-RA microspheres. As a control, stem cells will be cultured with daily addition of the same amounts of RA to the culture medium. Neuronal differentiation will be determined using neurite counting and Western blot analysis of neuronal markers expression. To optimize growth factors concentration in