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Labeling and Imaging of Human Mesenchymal Stem Cells with Quantum Dot Bioconjugates during Proliferation and Osteogenic Differentiation in Long Term

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Abstract—Quantum dots (QDs) are semiconductor nanocrystals that serve as promising alternatives to organic dyes for cell labeling. Because of their unique spectral, physical and chemical properties, QDs are useful for concurrently monitoring several intercellular and intracellular interactions in live normal cells and cancer cells over periods ranging from less than a second to over several days (several divisions of cells) [1]. Here, peptide CGGGRGD is immobilized on CdSe-ZnS QDs coated with carboxyl groups by cross linking with amine groups. These conjugates are directed by the peptide to bind with selected integrins on the membrane of human Mesenchymal stem cells. Upon overnight incubation with optimal concentration, QDs effectively labeled all the cells. Here, we report long-term labeling of human bone-marrow-derived mesenchymal stem cells (hMSCs) with RGD-conjugated QDs during self replication and differentiation into osteogenic cell lineages.

I. INTRODUCTION

Bioengineered tissues incorporating cultured cells should well mimic the substitute tissue. There is a critical need for long-term and reliable labeling of cells in tissue engineering to understand the relative contribution to the regenerated tissues and organs from delivered cells and host cells. Stem cells readily self-replicate and differentiate into lineage-specific cells, providing unique challenges for cell labeling. Reliable cell labeling tools can help delineate the fate and migration of delivered tissue-forming cells. An effective approach to track the implanted tissue-forming cells *in vivo* is to label the cells and thereby distinguish the labeled cells from the host cells.

Organic dyes such as rhodamine, fluorescein isothiocyanate (FITC), Alexa Fluor etc. and genetically encoded fluorescent proteins (GFPs) both have limitations as shown in Table I below [2], [3]. Quantum dots (QDs), a semiconductor nanocrystal such as cadmium selenium (CdSe) may circumvent many of the limitations of organic dyes and GFPs (Table I below).

Table I: Comparison of the Dyes

	Organic Dyes	GFPs	QDs
Emission Spectrum	Broad	Broad	Narrow
Excitation Spectrum	Narrow	Narrow	Broad
Photostability	No	Yes	Yes
Multiple cell labeling	No	No	Yes
pH sensitivity	Yes	No	No
Luminescence	Seconds	Hours	Months

The main objectives of this study were as follows:
(i) Conjugate RGD Peptide to Quantum Dot nanoparticles
(ii) Label human Mesenchymal stem cells during self replication with Quantum Dot (QD) bioconjugates
(iii) Determine cytocompatibility of QD-bioconjugates.
(iv) Explore QD labeling of human Mesenchymal stem cells during differentiation into lineage-specific cells.

II. MATERIALS AND METHODS

A. *Culture and expansion of human mesenchymal stem cells*

Human Mesenchymal stem cells (hMSCs) were cultured in growth medium consisting of Dulbecco's Modified Eagle's Minimal Essential Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin, Streptomycin.

B. *Preparation of Bioconjugated quantum dots*

Zinc Sulphide (ZnS) capped Cadmium Selenium (CdSe) Quantum Dots with functionalized carboxyl surface groups were obtained from Evident Technologies, Troy, NY. These QDs absorb light in the near UV spectrum (<450 nm) and emit in the orange spectrum (600 ± 10 nm).

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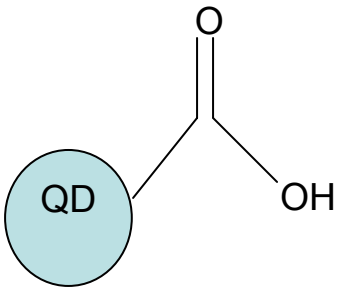

Fig 1: Structure diagram of ZnS capped CdSe quantum dots.

C. Conjugation of QDs to CGGGRGD peptide

QDs were then conjugated to the CGGGRGD peptide obtained from the Protein Research Laboratory at the University of Illinois at Chicago. The cystine (C) amino acid links to the CdSe-ZnS quantum dot through a thiol linkage, the GGG sequences of glycine (G) amino acids provide a spacer in the amino acid, and the RGD peptide goes and binds itself to specific integrins present on the cell surface.

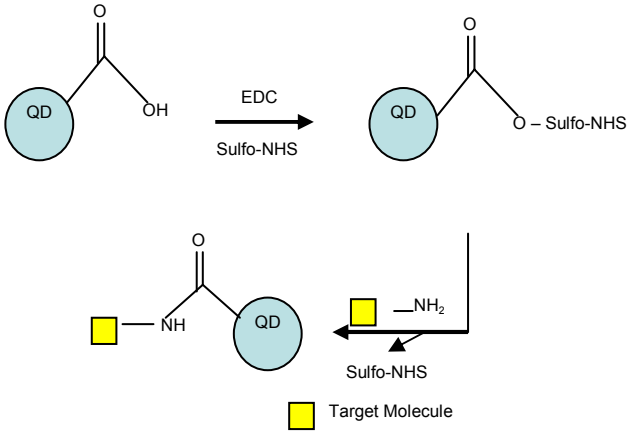

Fig. 2: The process of bioconjugation of RGD peptide on Zinc sulphide (ZnS) capped cadmium selenium (CdSe) quantum dots with functionalized carboxyl surface groups.

D. Brightfield and Fluorescence microscopy

Fluorescence microscopy was performed with an inverted microscope (Leica, Northvale, NJ) equipped with a mercury arc lamp (100 W) (Chiu Technology Corp., Kings Park, NY) and a 32007 Qdot 605 with 40 nm emission filter (Chroma, Rockingham, VT).

III. RESULTS

Optimal QD concentration: To determine the optimal labeling concentration, bioconjugated QDs were first diluted in DMEM medium to 0.5, 5, 20, and 50 nM concentrations. After 16-20 hrs of incubation, hMSCs were washed 5 times to remove unbound QDs and then placed in DMEM medium. Brightfield and fluorescence microscopy will be performed to determine the optimal QD concentration for labeling hMSCs.

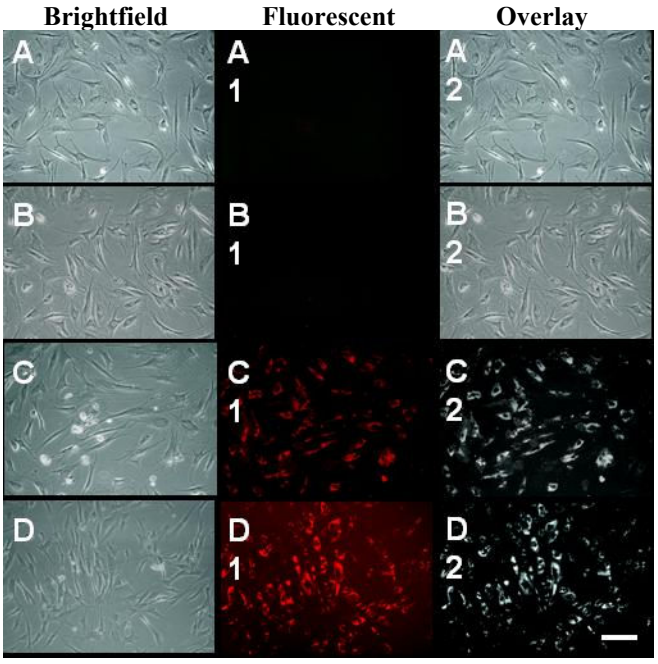

Fig. 3: Optimization of QD concentration. Scale bar 400 µm. A, A1, A2: 0.5 nM; B, B1, B2: 5 nM; C, C1, C2: 20 nM; D, D1, D2: 50 nM.

Optimal labeling time*:* To determine the optimal labeling time, hMSCs were incubated with bioconjugated QDs at a concentration of 30 nM for 5 min, 30 min, 2 hrs, and overnight (16-20 hrs).

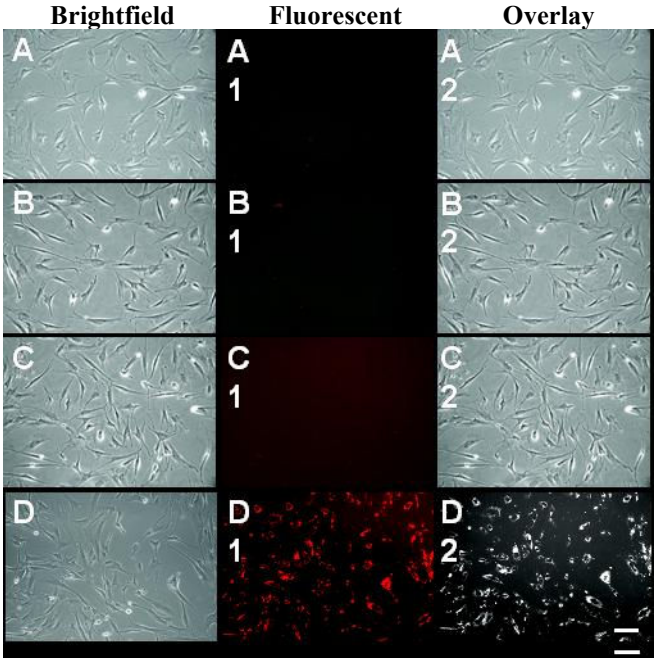

Fig 4: Optimization of labeling time. Scale bar: 400 µm. A, A1, A2: 5 min; B, B1, B2: 30 min; C, C1, C2: 2 hrs; D, D1, D2: Overnight (16-20) hrs.

QDs appeared to have labeled cytoskeletal structures but not the nucleus as seen in Fig 5.

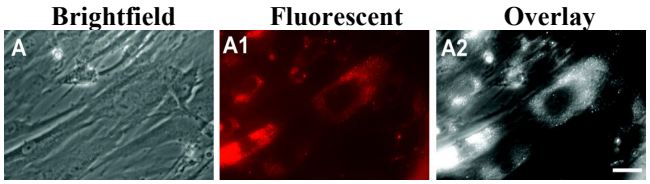

Fig. 5. High magnification (40X) image of hMSCs labeled overnight with 30 nM concentration of QDs. Scale bar 400 µm.

Fluorescent Activated Cell Sorting (FACS):

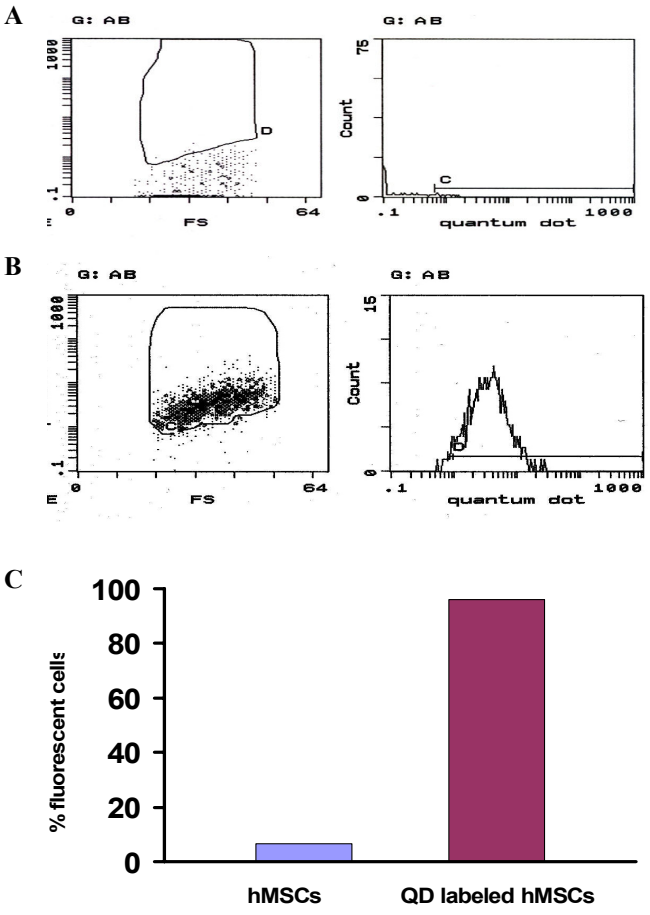

Fig. 6: Fluorescent activated cell sorting (FACS) of hMSCs with and without QD-biocongugate labels. A: FACS of hMSCs without QD labeling showing no fluorscence-activated cells, and low counting. B: FACS of hMSCs labeled with QD bioconjugates showing abundance of fluorescence-activated cells, and peak counting. C: Quantification of % fluorescent-activated cells with QD labels showing approximately 96% of hMSCs were QD-labeled, in contrast to 6% of hMSCs without QD label.

Effects of QD labeling on cell proliferation: To determine whether QD bioconjugates are cytotoxic, hMSCs were incubated with the bioconjugated QDs with an optimized 30 nM concentration and optimized incubation time of overnight (16-20 hrs) and continued to culture-expand QD labeled hMSCs for up to 22 days.

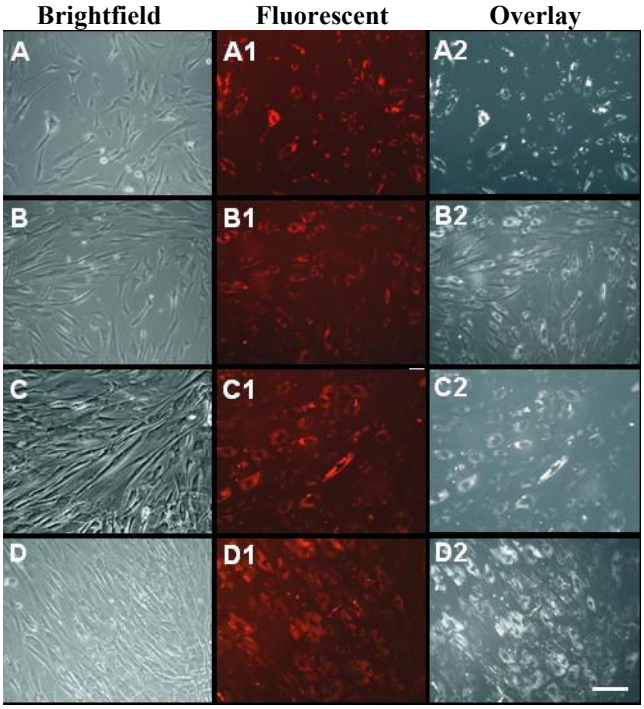

Fig 7. hMSCs continued to proliferate up to 22 days after QD labeling. Scale bar: 400 µm. A, A1, A2: overnight QD labeling; B, B1, B2: 4 days after QD labeling; C, C1, C2: 7 days after QD labeling; D, D1, D2: 22 days after QD labeling.

DNA Quantification: DNA content of the above cells proliferating in DMEM medium after incubation with QDs overnight at all time points was assessed using fluorescent quantification of Hoechst 33258 bound to double-stranded DNA (Fluorescent DNA Quantitation kit, Bio-Rad Labs, Hercules, CA).

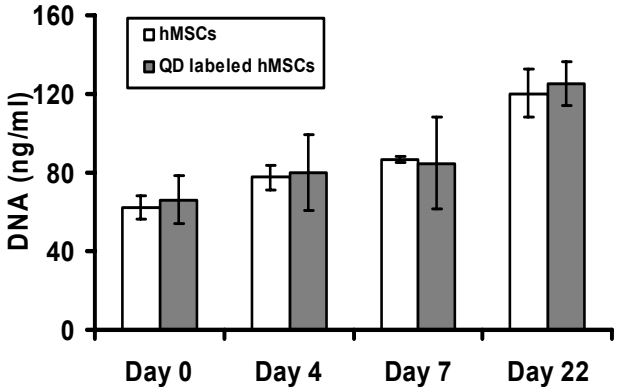

Fig. 8: DNA content of hMSCs with and without QD labeling.

Cell Viability: Cell viability of hMSCs labeled with QD bioconjugates was assessed using a Trypan Blue exclusion assay.

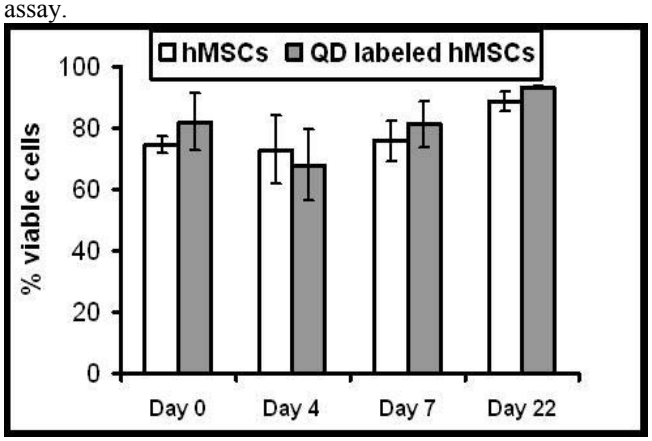

Fig. 9. Cell viability of hMSCs with or without labeling up to 22 days indicates cell survival after QD labeling.

Effects of QD labeling on cell differentiation: hMSCs were incubated with the bioconjugated QDs with an optimized 30 nM concentration and optimized incubation time of 16-20 hrs and then the hMSCs were treated with osteogenic supplements (OS) containing 100 nM dexamethasone, 0.05 mg/ml ascorbic acid, and 0.01 M β-glycerophosphate to the DMEM medium for up to 7 days.

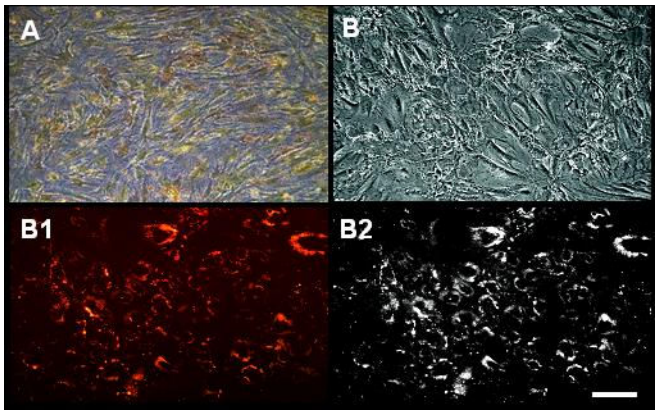

Fig. 10. hMSCs differentiated into osteogenic cells after QD labeling. A: ALP staining of osteoblasts B: Brightfield C: Fluorescent D: Overlay images. Scale bar 400 μm.

IV. CONCLUSION

The presently used QDs are cytocompatible with human mesenchymal stem cells (hMSCs). QD concentration and labeling time can be optimized for stem cells. The presently used QDs do not appear to interfere with self replication of human mesenchymal stem cells. Upon cell proliferation, the QD bioconjugates were present likely in both parent and daughter hMSCs. Neither do QDs interfere with osteogenic differentiation of hMSCs. The presently used QDs are safe to label human mesenchymal stem cells for in vitro use. Although QD bioconjugates have recently been used to label a number of cells types such as malignant cell lines, endothelial cells, erythrocytes, and fibroblasts cells, the present data provide original demonstration that QD-labeled bioconjugates are capable of labeling stem cells during their self replication and differentiation processes.

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